

ANALYSIS OF POTENT ODORANTS IN SPEARMINT OILS

BY

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THESIS

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ABSTRACT

Spearmint has been grown in gardens since the 9th century as an herb thought to possess a wide range of health benefits. Spearmint was introduced to the Massachusetts Bay Colony in 1628. Commercial cultivation of spearmint in the United States began in the 1790's. The production of spearmint spread west and now is primarily grown in Washington and Oregon. The majority of the world's spearmint is grown in the United States, with production totaling 2.93 million pounds in 2013. The popularity of spearmint is attributed mainly to its use in chewing gum and breath mints, as well as other confectionery products and oral healthcare products. While the major volatile constituents of spearmint are well known, there has been little research on the potent odorants in spearmint. The identification and quantitation of these compounds was performed in this study to give a better understanding the flavor chemistry of spearmint oils.

Potent odorants in Native spearmint, Scotch spearmint, and Macho mint oils were identified by application of gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). Aroma extract dilution analysis was performed to determine the potency of the odorants. Of the 85 odorants detected, R-(-)-carvone was the most potent odorant in all three spearmint oils. Eugenol, ethyl-2-methyl butyrate, β -damascenone, and (3*E*,5*Z*)-1,3,5-undecatriene were also identified to be predominant odorants in the spearmint oils. New potent odorants not previously identified in spearmint include 1-hexen-3-one, 3-methyl-2-butene-1-thiol, and 2-methylisoborneol.

Forty-six compounds in Native spearmint, Scotch spearmint, and Macho mint oils were quantified using various methods. Nineteen high abundance compounds were quantified using a gas chromatography-flame ionization detector (GC-FID), 20 were quantified by stable isotope

dilution analysis (SIDA), and 14 were quantified by gas chromatography-olfactometry (GC-O) dilution analysis. Seven of the compounds quantified by GC-O dilution analysis were also quantified by SIDA. The concentration results were used to calculate the odor activity value (OAV) of each compound by dividing the concentration by the odor detection threshold of the compound in water. Among the compounds quantified, those with the highest OAVs were R-(-)-carvone, 1,8-cineole, (*E,Z*)-2,6-nonadienal, β -damascenone, and (*3E,5Z*)-1,3,5-undecatriene.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES.....	vi
CHAPTER ONE: INTRODUCTION.....	1
1.1 References.....	4
CHAPTER TWO: LITERATURE REVIEW.....	5
2.1 Spearmint.....	5
2.2 Flavor chemistry of spearmint.....	7
2.3 Aroma analysis techniques.....	9
2.4 Figures and Tables.....	12
2.5 References.....	14
CHAPTER THREE: IDENTIFICATION OF POTENT ODORANTS IN SPEARMINT OILS.....	15
3.1 Abstract.....	15
3.2 Keywords.....	15
3.3 Introduction.....	15
3.4 Materials and methods.....	17
3.5 Results and discussion.....	20
3.6 Figures and tables.....	24
3.7 References.....	30
CHAPTER FOUR: QUANTITATION OF POTENT ODORANTS.....	32
4.1 Abstract.....	32
4.2 Keywords.....	32
4.3 Introduction.....	32
4.4 Materials and methods.....	34
4.5 Results and discussion.....	41
4.6 Figures and tables.....	44
4.7 References.....	51
CHAPTER FIVE: SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH.....	54
5.1 Figures and tables.....	58
APPENDIX A: Quantity of labeled isotopes for stable isotope dilution analysis.....	59
APPENDIX B: Quantification data.....	60
APPENDIX C: Calibration curves.....	68
APPENDIX D: GC-O dilution analysis quantitation data.....	88

LIST OF FIGURES AND TABLES

CHAPTER TWO: LITERATURE REVIEW

Table 2.1	Composition of spearmint oil.....	12
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CHAPTER THREE: IDENTIFICATION OF POTENT ODORANTS IN SPEARMINT OILS

Table 3.1	Aroma-active compounds detected in spearmint oils.....	24
Table 3.2	Flavor dilution factors of compounds detected in spearmint oils.....	27
Table 3.3	Odorants previously identified in spearmint oils.....	29

CHAPTER FOUR: QUANTITATION OF POTENT ODORANTS

Figure 4.1	Chemical structures of isotope standards.....	44
Table 4.1	Analytes, labeled isotopes, selection ions, and response factors used for SIDA.....	46
Table 4.2	Concentrations of compounds quantified by SIDA.....	47
Table 4.3	Concentrations of compounds quantified by GC-FID.....	48
Table 4.4	Concentrations of compounds quantified by GC-O dilution analysis.....	49
Table 4.5	Concentrations, odor detection thresholds, and odor activity values for selected odorants in spearmint oils.....	50

CHAPTER FIVE: SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH

Figure 5.1	Flow diagram of quantitation method selection.....	58
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CHAPTER ONE

INTRODUCTION

Spearmint is believed to have originated in the Mediterranean. The first written record of spearmint dates back to the 9th century in Europe, where it was grown in convent gardens. The popularity of spearmint in Europe grew with the increase in claims about its perceived health benefits. The commercial cultivation of spearmint began in England during the mid-1700's. English colonists brought spearmint root stocks to the Massachusetts Bay Colony shortly after its foundation in 1628. After its introduction, spearmint quickly spread across New England. The cultivation of spearmint in the United States began in the 1790's in western Massachusetts. In 1846, spearmint cultivation moved west to New York, where growing conditions were more favorable. Thirty years later, spearmint production began in Michigan. The popularity of spearmint increased in the 1890's due to its use in chewing gum and toothpaste, and with the discovery of a new, rare variety of spearmint in Wisconsin. The new spearmint variety was thought to have originated in Scotland and thus it was named Scotch spearmint. This new plant was found to be more tolerant to frost and to have a higher oil yield than Native spearmint. By the early 1900's, mint distilleries had been set up in southern Washington and the Willamette Valley in northern Oregon. In the Midwest, the fungal disease *Verticillium* wilt had decimated much of the mint crops by 1950. Due to the devastation in the Midwest, the Farwest gained prominence as a spearmint growing region (Landing, 1969).

Native and Scotch spearmint are currently grown in both the Midwest and Farwest, with the Farwest being the prominent spearmint growing region. Approximately 2.93 million pounds of spearmint oil was produced in the United States in 2013. Spearmint was grown across 24,500 acres, equating to approximately 119 pounds of spearmint oil per acre (Anon, 2014). Spearmint

is currently used for confectionery products such as chewing gum and breath mints and oral health products like toothpaste, mouthwash, and dental floss (Lawrence, 2007).

The main volatile compounds in Native and Scotch spearmint oils are well known. The major constituent of both is R-(-)-carvone, which has a distinctive spearmint-like aroma. Other volatiles in high abundance are limonene, 1,8-cineole, and myrcene. Studies have also shown that the composition of spearmint oils vary among growing regions, seasons, and within varieties or species (Lawrence, 2007).

While the volatiles in spearmint are well-characterized, only two studies in the past literature have directly analyzed the odorants in Native spearmint oil. Jirovetz et. al. (2002) studied the odor-active compounds in Native spearmint grown near Ngaoundéré, Cameroon. They used steam distillation to attain the spearmint oil from the plants, and extracted the Native spearmint oil with dichloromethane. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in combination with solid phase micro-extraction (SPME) were implemented in the identification of compounds in the Native spearmint oil. To identify the odorants in Native spearmint, they used a “GC-sniffing technique”, more commonly known as GC-olfactometry (GC-O). The researchers based the intensity of the individual odorants on the description of the aroma of the Native spearmint oil given by perfumers. From the perfumers’ impressions, the researchers attested that the most potent odorants in the Native spearmint oil were (-)-carvone, (-)-limonene, and 1,8-cineole. Other important compounds were hexanal (green), linalool and linalyl acetate (floral), 3-methylbutanal and 2-methylbutanal (fruity), and Z- β -ocimene and *E*- β -ocimene (spicy) (Jirovetz et. al., 2002).

In the second study on the odorants in Native spearmint, Diaz-Maroto et. al. (2008) examined volatiles isolated from Native spearmint grown in Spain using simultaneous distillation-solvent extraction. The researchers used GC-MS and GC-O with Supelco SPB-1 and BP-21 columns to determine the composition of the mint extracts. Five panelists rated the aromas by GC-O based on intensity using a scale from one to five. Their research showed that R-(-)-carvone was the most intense odorant in the Native spearmint extracts. They indicated that other important odorants were 1,8-cineole and cis-dihydrocarveol, which both had minty aromas, ethyl 3-methylbutanoate (fruity and sweet), methional (baked potato), 2,5-diethyltetrahydrofuran (toasty, roasted nuts), 1-octen-3-ol and 1-octen-3-one (mushroom), *trans*-2-nonenal (cucumber), β -bourbonene (fruity), and *epi*-bicyclosesquiphellandrene (fruity, peach). They also noted the some of the odorants were present in low concentrations or did not show a GC peak (Diaz-Maroto et. al., 2008).

The major volatile constituents of Native spearmint and Scotch spearmint are well known. However, there has been little research on the trace potent odorants in Native spearmint and no research on the odorants in Scotch spearmint. Furthermore, it is hypothesized that there are trace level odorants, both known and unknown, that contribute to the aroma of spearmint. The identification and quantitation of these compounds will give a better understanding of spearmint oils. The first objective of this research was to identify the potent odorants in spearmint oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. The second objective was to develop methods to accurately and precisely quantify these trace-level potent odorants.

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CHAPTER TWO

LITERATURE REVIEW

2.1 SPEARMINT

Spearmint is a member of the taxonomic family Lamiaceae, which is more commonly known as the Mint Family. There are two main varieties of spearmint: Native spearmint (*Mentha spicata*) and Scotch spearmint (*Mentha x gracilis*). Native spearmint is considered one of the five main mint varieties, while all other mint varieties are either hybrids, cultivars, or subspecies of other mints. Scotch spearmint is a hybrid between Native spearmint and cornmint (*Mentha arvensis*) (Lawrence, 2007). One important cultivar of spearmint is Macho mint (*Mentha spicata* CV. Macho Mint). This spearmint cultivar was discovered in 1979 in Blue Eye, Missouri and is noted for its large size in comparison to other spearmint plants (Long, 2010). Spearmint is a perennial herb with violet flowers and can grow more than three feet tall. Its serrated leaves have a distinctive pungent mint flavor (Lawton, 2002).

Spearmint is believed to have originated in the Mediterranean. The first written record of spearmint dates back to the 9th century in Europe, where it was grown in convent gardens. The popularity of spearmint in Europe grew with the increase in claims about its perceived health benefits. The commercial cultivation of spearmint began in England during the mid-1700's. English colonists brought spearmint root stocks to the Massachusetts Bay Colony shortly after its foundation in 1628. After its introduction, spearmint quickly spread across New England. The cultivation of spearmint in the United States began in the 1790's in western Massachusetts. In 1846, spearmint cultivation moved west to New York, where growing conditions were more favorable. Thirty years later, spearmint production began in Michigan. The popularity of spearmint increased in the 1890's due to its use in chewing gum and toothpaste, and with the

discovery of a new, rare variety of spearmint in Wisconsin. The new spearmint variety was thought to have originated in Scotland and thus it was named Scotch spearmint. This new plant was found to be more tolerant to frost and to have a higher oil yield than Native spearmint. By the early 1900's, mint distilleries had been set up in southern Washington and the Willamette Valley in northern Oregon. In the Midwest, the fungal disease *Verticillium* wilt had decimated much of the mint crops by 1950. Due to the devastation in the Midwest, the Farwest gained prominence as a spearmint growing region (Landing, 1969).

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Spearmint is commercially grown by planting rootstocks of the plant rather than seeds, since mint plants are functionally sterile. Spearmint is perennial and will typically last around 10 years of production. Spearmint harvest occurs in early to mid-August, when the spearmint oil quality and yield are at their highest. After spearmint is harvested, distillation is used to obtain the essential oil. Mint distillation happens in the mint fields. The cut spearmint plants are transferred to a large metal distillation tub on the back of a truck. When the tub is full, it is fitted with a steam-tight lid containing an outlet pipe. Steam from a boiler is then piped into the bottom of the distillation tub. Steam condenses on the surface of the spearmint and causes the spearmint oil to vaporize. The vaporized spearmint oil is passed through the outlet pipe on the top of the

tub and sent to a condenser, where the oil is cooled back to a liquid. The spearmint oil can be easily separated from the remaining water, as the two liquids are immiscible (Lawrence, 2007).

2.2 FLAVOR CHEMISTRY OF SPEARMINT

The main volatile compounds in Native and Scotch spearmint oils are well known. The major constituent of both is R-(-)-carvone, which has a distinctive spearmint-like aroma. Other volatiles in high abundance are limonene, 1,8-cineole, and myrcene. All of the main constituents of spearmint oil are detailed in Table 2.1. Studies have also shown that the composition of spearmint oils vary among growing regions, seasons, and within varieties or species (Lawrence, 2007).

While the volatiles in spearmint are well-characterized, only two studies in the past literature have directly analyzed the odorants in Native spearmint oil. Jirovetz et. al. (2002) studied the odor-active compounds in Native spearmint grown near Ngaoundéré, Cameroon. They used steam distillation to attain the spearmint oil from the plants, and extracted the Native spearmint oil with dichloromethane. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in combination with solid phase micro-extraction (SPME) were implemented in the identification of compounds in the Native spearmint oil. To identify the odorants in Native spearmint, they used a “GC-sniffing technique”, more commonly known as GC-olfactometry (GC-O). The researchers based the intensity of the individual odorants on the description of the aroma of the Native spearmint oil given by perfumers. From the perfumers’ impressions, the researchers attested that the most potent odorants in the Native spearmint oil were (-)-carvone, (-)-limonene, and 1,8-cineole. Other important compounds were hexanal

(green), linalool and linalyl acetate (floral), 3-methylbutanal and 2-methylbutanal (fruity), and (*Z*)- β -ocimene and (*E*)- β -ocimene (spicy) (Jirovetz et. al., 2002).

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A third study identified and quantified off-notes in Native spearmint, Scotch spearmint, and peppermint oils to distinguish ‘good oils’ from those with off-notes. Coleman et. al. (2002) first employed a trained panel to distinguish ‘good’ mint oils from those with off-notes. Then they used SPME in combination with GC-MS equipped with a polar DBWAXETR column to analyze the mint oils. The researchers quantified the major constituents in the ‘good’ mint oils based on the peak area percentages. The compound concentrations were divided by the odor threshold for each compound. They used these values to determine the top ten influential compounds in the odor profile of mint oils. The researchers reported that 1,8-cineole had the highest contribution to the aroma of both Native spearmint and Scotch spearmint oils. They

listed the next most important compounds to spearmint aroma as R-(-)-carvone and 2-methylbutanal. The concentrations of the ten selected compounds were calculated in the mint oils with off-notes and compared to the concentrations from the ‘good’ mint oils (Coleman et. al., 2002).

Carvone is one of many monoterpenes present in spearmint oils. Monoterpene biosynthesis begins with geranyl diphosphate. Cyclase enzymes isomerize geranyl diphosphate to linalyl pyrophosphate, which is then cyclized to form (-)-limonene. In spearmint plants, the enzyme (-)-limonene-6-hydroxylase hydroxylates limonene at the C₆ position to produce (-)-trans-carveol, which is then oxidized to form (-)-carvone. From there, (-)-carvone can undergo various oxidation and reduction reactions to form isomers of dihydrocarvone, dihydrocarveol, carvyl acetate, and dihydrocarvyl acetate. Other monoterpenes in spearmint are formed from from the hydroxylation of (-)-limonene by (-)-limonene-6-hydroxylase or synthases that work on linalyl diphosphate (Lawrence, 2007).

2.3 AROMA ANALYSIS TECHNIQUES

The aroma profile of a food is the result of the volatile compounds within the food. Volatiles can reach the olfactory receptors in one’s nose either orthonasally, when a food is sniffed, or retronasally, when the food is being chewed. All aroma-active compounds are volatile, yet not all volatile compounds are aroma-active. Each compound has a different odor detection threshold. Compounds with a high odor threshold are difficult to smell even in high concentrations. However, compounds with very low odor thresholds only require the compound to be present in small amounts to be noticeable (Reineccius, 2006).

Volatile compounds can be analyzed using gas chromatography-mass spectrometry (GC-MS). While GC-MS allows for the identification of volatiles in a sample, it provides no

indication as to which compounds are odor active. To determine which individual compounds are odor active in a sample, one needs to smell each compound. This can be achieved by using gas chromatography-olfactometry (GC-O). In this method of analysis, volatile compounds are separated by a GC column and are then sent to the olfactory port where one can smell the compounds individually. The aroma, retention time, and intensity of the odorants are noted while one analyzes a sample using GC-O. The retention times of the odorants are compared to the retention times of *n*-alkanes to give a retention index (RI) for each odorant (van den Dool and Kratz, 1963). The RI values, in combination with aroma descriptions, can be compared to RI values and aroma descriptions from literature and GC-MS results to give tentative compound identifications.

Not only can GC-O be used to identify odorants in a sample, it can also be used to determine the relative potencies of the odorants. It is important to find the odorant potencies as it gives an indication of the compounds which are most important to the aroma of a sample. The relative odorant potencies can be calculated using a technique called aroma extract dilution analysis (AEDA). AEDA involves the analysis of serial dilutions of a sample by GC-O to determine the flavor dilution (FD) factor of each compound. The FD factor is the highest dilution at which an odorant is detected. A compound with a high FD factor is more potent in a sample than a compound with a low FD factor (Grosch, 1993).

GC-O completely volatilizes compounds, so AEDA results only indicate the potency of the individual compounds in air. For this reason, GC-O does not account for interactions among the compounds or the matrix in which they exist. To determine the contribution of an odorant within a certain matrix, an odor activity value (OAV) is calculated. The OAV of a compound is

found by dividing its odor threshold in a sample matrix by its concentration in the product. (Grosch, 1993).

To find the concentrations of odorants in the spearmint oils, several quantitation methods can be implemented. Stable isotope dilution analysis (SIDA) involves using either a deuterium or carbon-13 labeled isotope of the target analyte as an internal standard to determine the concentration of that compound. SIDA gives precise and accurate quantitation results since a labeled isotope is nearly identical to the target analyte, and therefore the isotope is a perfect internal standard. However, for trace potent odorants that are not detectable by GC-MS, SIDA is not a viable option. GC-O dilution analysis can be used to determine the concentrations of trace compounds with low odor thresholds by comparing the flavor dilution (FD) factor of a compound with a known concentration to the FD factor of that compound in a sample. Additionally, SIDA is not a practical method to determine the concentration of compounds in high abundance. Quantitation using a GC-FID can be used to quantify high abundance compounds by comparing their peak areas with that of an internal standard.

2.4 FIGURES AND TABLES

Table 2.1 Composition of spearmint oil^a

Compound	IUPAC Name	CAS Number	Percent in Spearmint Oil
3-methylbutanal	3-methylbutyraldehyde	590-86-3	0-0.1%
α -pinene	(1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	80-56-8	0.2-0.9%
α -thujene	1-isopropyl-4-methylbicyclo[3.1.0]hex-3-ene	2867-05-2	0-0.7%
<i>trans</i> -2,5-diethyltetrahydrofuran	[2R,5R,(-)]-2,5-diethyltetrahydrofuran	32101-31-8	0-0.1%
β -pinene	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane	127-91-3	0.7-0.8%
sabinene	4-methylene-1-(1-methylethyl)bicyclo[3.1.0]hexane	3387-41-5	0-0.5%
myrcene	7-methyl-3-methylene-1,6-octadiene	123-35-3	1.5-4.4%
α -terpinene	4-methyl-1-(1-methylethyl)-1,3-cyclohexadiene	99-86-5	0-0.2%
limonene	1-methyl-4-(1-methylethenyl)-cyclohexene	138-86-3	8.7-11.6%
1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane	470-82-6	0-2.2%
(Z)- β -ocimene	(3Z)-3,7-dimethyl-1,3,6-octatriene	3338-55-4	0-0.2%
γ -terpinene	4-methyl-1-(1-methylethyl)-1,4-cyclohexadiene	99-85-4	0-0.5%
(E)- β -ocimene	(3E)-3,7-dimethyl-1,3,6-octatriene	3779-61-1	0-0.1%
<i>p</i> -cymene	1-methyl-4-(1-methylethyl)benzene	99-87-6	0-0.4%
terpinolene	1-methyl-4-(1-methylethylidene)-cyclohexene	586-62-9	0-0.1%
3-octyl acetate	octan-3-yl acetate	4864-61-3	0-0.4%
(Z)-3-hexenol	(Z)-hex-3-en-1-ol	928-96-1	0-0.1%
3-octanol	octan-3-ol	589-98-0	0-1.5%
1-octen-3-ol	oct-1-en-3-ol	3391-86-4	0-0.9%
menthone	(2S,5R)-2-isopropyl-5-methylcyclohexanone	14073-97-3	0-1.2%
<i>trans</i> -sabinene hydrate	(1S,4R,5R)-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-4-ol	17699-16-0	0.4-2.2%
isomenthone	5-methyl-2-propan-2-ylcyclohexan-1-one	36977-92-1	0-0.1%
β -bourbonene	1-methyl-5-methylidene-8-(propan-2-yl)tricyclo[5.3.0.0 ^{2,6}]decane	5208-59-3	0-1.9%
α -copaene	1R,2S,6S,7S,8S)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.0 ^{2,7}]dec-3-ene	3856-25-5	0-0.1%
linalool	3,7-dimethylocta-1,6-dien-3-ol	78-70-6	0-0.1%
<i>cis</i> -sabinene hydrate	(1R,2S,5S)-5-isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	15826-82-1	0-0.1%

Table 2.1 continued on next page.

Table 2.1 (continued)

Compound	IUPAC Name	CAS Number	Percent in Spearment Oil
β -caryophyllene	4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	87-44-5	0-1.9%
terpinen-4-ol	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	562-74-3	0-1.4%
<i>cis</i> -dihydrocarvone	(2S,5R)-5-(1-methylethenyl)-2-methylcyclohexanone	6909-25-7	0-2.5%
<i>trans</i> -dihydrocarvone	(2R,5R)-5-(1-methylethenyl)-2-methylcyclohexanone	5948-04-9	0-0.2%
γ -muurolene	(1R,4aR,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	30021-74-0	0-0.4%
(<i>E</i>)- β -farnesene	7,11-dimethyl-3-methylene-1,6,10-dodecatriene	18794-84-8	0-0.7%
dihydrocarvyl acetate	(2-methyl-5-prop-1-en-2-ylcyclohexyl) acetate	20777-49-5	0-3.0%
α -terpineol	2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol	98-55-5	0-0.9%
germacerene D	(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	37839-63-7	0-1.5%
neodihydrocarveol	(1R,2S,5S)-2-methyl-5-(prop-1-en-2-yl)cyclohexanol	18675-33-7	0-0.6%
carvone	2-methyl-5-(1-methylethenyl)-2-cyclohexenone	6485-40-1	59.3-70.0%
dihydrocarveol	(1R,2R,5R)-2-methyl-5-prop-1-en-2-ylcyclohexan-1-ol	20549-47-7	0-2.4%
neoisodihydrocarveol	(1R,2S,5R)-2-methyl-5-prop-1-en-2-ylcyclohexan-1-ol	53796-80-8	0-1.6%
<i>cis</i> -carvyl acetate	[(1R,5R)-2-methyl-5-prop-1-en-2-yl-1-cyclohex-2-enyl] acetate	1205-42-1	0-0.6%
<i>trans</i> -carvyl acetate	[(1R,5S)-2-methyl-5-prop-1-en-2-yl-1-cyclohex-2-enyl] acetate	1134-95-8	0-0.7%
<i>trans</i> -carveol	(1S,5R)-2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol	1197-07-5	0-0.4%
<i>cis</i> -carveol	(1R,5R)-2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol	1197-06-4	0-0.2%
(<i>Z</i>)-jasmone	3-methyl-2-[(<i>Z</i>)-pent-2-enyl]cyclopent-2-en-1-one	488-10-8	0-0.3%
viridiflorol	(1aR,4S,4aS,7R,7aS)-1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulen-4-ol	552-02-3	0-0.4%

^aFrom Lawrence, 2007.

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CHAPTER THREE

IDENTIFICATION OF POTENT ODORANTS IN SPEARMINT OILS

3.1 ABSTRACT

Potent odorants in Native spearmint, Scotch spearmint, and Macho mint oils were identified by application of gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). Aroma extract dilution analysis was performed to determine the relative potency of the odorants. Of the 85 odorants detected, R-(-)-carvone was the most potent odorant in all three spearmint oils. Eugenol, ethyl-2-methyl butyrate, β -damascenone, and (3E,5Z)-1,3,5-undecatriene were also identified to be predominant odorants in the spearmint oils. New potent odorants not previously identified in spearmint include 1-hexen-3-one, 3-methyl-2-butene-1-thiol, and 2-methylisoborneol.

3.2 KEYWORDS

Spearmint, essential oil, gas-chromatography-olfactometry, gas chromatography-mass spectrometry, aroma extract dilution analysis

3.3 INTRODUCTION

Spearmint has been grown in gardens since the 9th century as an herb thought to possess a wide range of health benefits. Spearmint was introduced to the Massachusetts Bay Colony in 1628. Commercial cultivation of spearmint in the United States began in the 1790's. The production of spearmint spread west and now is primarily grown in Washington and Oregon. The majority of the world's spearmint is grown in the United States, with production totaling 2.93 million pounds in 2013. The popularity of spearmint is attributed mainly to its use in chewing gum and breath mints, as well as other confectionery products and oral healthcare products.

It is well documented that R-(-)-carvone is the primary odorant contributing to the flavor of spearmint. Many studies have analyzed the composition of the major constituents of Native and Scotch spearmint essential oils, yet only two studies have characterized the odor-active compounds in these oils. In the first study, Jirovetz et. al. (2002) indicated the most potent odorants in Native spearmint oil were R-(-)-carvone, limonene, and 1,8-cineole. In the second study, Diaz-Maroto et. al. (2008) reported that the most potent odorants in Native spearmint oil were R-(-)-carvone, 1,8-cineole, and *cis*-dihydrocarveol. While these studies provide some insight as to the potent odorants in spearmint oil, additional research is needed to provide a more complete understanding of the complex flavor of spearmint oil. Furthermore, research has only been performed on the odorants in Native spearmint oil; the odorants in Scotch and other varieties of spearmint oil have never been reported.

The use of gas-chromatography-olfactometry (GC-O) is essential for the determination of odor-active compounds in a sample. GC-O makes use of an olfactory port which allows for a person to smell well-separated compounds, i.e., essentially pure odorants, as they are eluted from a GC. Additionally, GC-O is useful for establishing the relative potency of the odorants in the sample. Aroma extract dilution analysis (AEDA) involves the determination of odorant potency by the analysis of a serial dilution series of a sample or aroma extract by GC-O. The flavor dilution (FD) factor of a compound is the highest dilution at which an odorant is detected by AEDA and indicates the potency of that odorant within a sample. A compound with a high FD factor is more potent than a compound with a low FD factor (Grosch, 1993).

In this study, Native spearmint, Scotch spearmint, and Macho mint oils were analyzed using GC-O and GC-MS to identify odor-active compounds, with an emphasis on the identification of trace potent odorants. The odorant potency of each compound was determined

using AEDA. The results of this study provide a better understanding of the flavor of spearmint oils.

3.4 MATERIALS AND METHODS

Materials

Farwest Native spearmint (crop year 2011, manufactured May 3, 2013), Farwest Scotch spearmint (crop year 2012, manufactured September 3, 2012), and Macho mint oil were obtained from the Wm. Wrigley Jr. Company (Chicago, IL).

Chemicals

n-Alkane standards and ethyl acetate (99.5%) were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO). Diethyl ether (anhydrous, 99.9%) was acquired from Thermo Fisher Scientific Inc. (Waltham, MA). Liquid nitrogen, ultra-high purity (UHP) nitrogen gas, UHP helium, and UHP hydrogen were purchased from S.J. Smith Co. (Davenport, IA).

Reference standard compounds. The following authentic reference standards used to confirm the retention indices and mass spectra of the odor-active compounds listed in Table 3.1 were acquired from Sigma-Aldrich Co. LLC: (*E,Z*)-2,6-nonadienal, phenylacetaldehyde, isovaleric acid, eugenol, vanillin, β -ionone, β -damascenone, *p*-cresol, myrcene, methyl-2-methylbutyrate, ethyl-2-methylbutyrate, ethyl-3-methylbutyrate, menthone, menthol, R-(-)-carvone, carvyl acetate (*Z/E* mix), linalool, limonene, hexanal, octanal, nonanal, dimethyl sulfide, dimethyl trisulfide, methional, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and 2-phenylethanol. 1,3,5-Undecatriene was purchased from Bedoukian Research, Inc. (Danbury, CT). 1-Hexen-3-one and 1-octen-3-one were acquired from Alfa Aesar (Ward Hill, MA).

Syntheses

3-Methyl-2-butene-1-thiol was synthesized as previously reported for butylthiol, with 3,3-dimethylallyl bromide (Sigma Aldrich, St. Louis, MO) being substituted for *n*-butyl bromide (Ishikawa et. al., 1984). MS-EI, *m/z* (intensity in %): 41(100), 69 (58), 102 (33, M^+), 68 (28), 39 (22), 53 (16), 67 (10), 45 (8), 59 (5), 47 (5).

Identification of Aroma-Active Compounds

Gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS) were implemented for the identification of odor-active compounds in the spearmint oils.

Gas Chromatography-Olfactometry. The spearmint oils were analyzed by three people using gas-chromatography-olfactometry (GC-O) to determine the aroma-active compounds in the spearmint oils. The GC-O system consisted of an Agilent HP 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a flame ionization detector (FID), a flame photometric detector (FPD), and an olfactory detection port (ODP, DATU Technology Transfer, Geneva, NY). Analyses were performed on both an RTX-WAX column (15 m x 0.53 mm ID x 1 μ m; Restek Corporation, Bellefonte, PA) and an RTX-5 column (15 m x 0.53 mm ID x 1 μ m; Restek Corporation). The spearmint oils (2 μ L) were injected by cold split mode (200:1 split ratio; initial inlet temperature -50 °C, held 0.10 min, and then ramped at 12 °C/s to 250 °C with final hold time of 20 min) using a CIS4 inlet (Gerstel, Mülheim, Germany). The oven temperature was held at 40 °C for 5 minutes, ramped to 225 °C at 8 °C/min, and held at 225 °C for 30 minutes. Helium was used as the carrier gas with a constant flow rate of 18.4 mL/min. The eluent from the column was divided using deactivated fused silica tubing (1 m x 0.25 mm ID x 0.5 μ m; Restek Corporation, Bellefonte, PA) and sent to the FID, FPD, and ODP. The temperature for all three detectors was 250 °C.

Gas Chromatography-Mass Spectrometry. GC-MS was used to identify volatile compounds in the spearmint oils. The GC-MS system consisted of a 6890 GC-HP 5973N mass selective detector (Agilent Technologies, Inc.). Analyses were performed on both a Stabliwax (30 m x 0.25 mm ID, 0.25 μ m; Restek Corporation) and a SAC-5 column (30 m x 0.25 mm ID, 0.25 μ m; Sigma-Aldrich Co. LLC.). The spearmint oils (2 μ L) were injected by cold split mode (200:1 split ratio; initial inlet temperature -50 °C, held 0.10 min, and then ramped at 12 °C/s to 250 °C with final hold time of 20 min) using a CIS4 inlet (Gerstel). The oven temperature was held at 40 °C for 5 minutes, ramped to 225 °C at a rate of 4 °C/min, and held at 225 °C for 30 minutes. Helium was used as the carrier gas with a flow rate of 0.8 mL/min. The temperature of the mass spectrometer was 250 °C, and the mass scan range was 35 to 300 m/z. The ionization energy for the mass spectrometer was 70 eV. The chromatographs were analyzed with MSD ChemStation Enhanced Data Analysis Software (Agilent Technologies, Inc.). The mass spectrum for each peak was compared to the National Institute of Standards and Technology (NIST) 2008 Mass Spectral Library.

The retention indices (RIs) were determined on both a RTX-WAX and RTX-5 column for each compound. A retention index was determined by comparing the retention time of the compound to the retention times of *n*-alkanes, as described by van den Dool and Kratz (1963). The RI values, in combination with aroma descriptions and mass spectral library results, were compared with literature values and online databases (Flavornet and Pherobase) to give tentative compound identifications. Authentic standards of tentatively identified compounds were analyzed by GC-O to confirm their retention indices and aromas for the purpose of positive identification.

Aroma Extract Dilution Analysis

Aroma extract dilution analysis (AEDA) was used to determine the potency of the odor-active compounds in the spearmint oils. Serial dilutions (1:2 v/v) of each mint sample were made by diluting the mint oil with ethyl acetate. Starting with the 1:8 dilution, the dilutions were analyzed sequentially by GC-O as previously described using an RTX-WAX column (15 m x 0.53 mm ID x 1 μ m; Restek Corporation) until the only odorant detectable was (-)-carvone. The highest dilution at which an odorant was detected was considered the flavor dilution (FD) factor for that compound within each sample dilution series.

3.5 RESULTS AND DISCUSSION

Identification of Aroma-Active Compounds

A combined total of eighty-five compounds were detected in all three spearmint oils, as shown in Table 3.1. Sixty-nine compounds were detected in Native spearmint oil, 68 compounds were detected in Scotch spearmint oil, and 74 compounds were detected in the Macho mint oil. Of these compounds, 41 were positively identified, four were tentatively identified, and 40 odorants remained unknown.

As seen in Table 3.3, eight compounds were identified which had not been previously reported in spearmint, including 1-hexen-3-one (plastic), 3-methyl-2-butene-1-thiol (skunky), and 2-methylisoborneol (earthy). Of the 38 odorants positively identified, 20 had been reported spearmint, but had never been identified as contributing to spearmint aroma. Some of these compounds include ethyl-2-methylbutyrate, (3*E*,5*Z*)-1,3,5-undecatriene, and eugenol, which are among the most potent odorants identified in this study.

While R-(-)-carvone and most of the major constituents of spearmint oil are formed from monoterpene biosynthesis, the trace potent odorants are formed via several other pathways. One of the most potent odorants in spearmint oil, eugenol, is formed from the thermal decomposition of lignin in the spearmint plants during steam distillation. This reaction is also the source of other potent odorants in spearmint oil, such as 4-vinylguaiacol, *p*-cresol, and vanillin (Faix et. al., 1990). Steam distillation of spearmint also causes the formation of trace potent odorants in a process called Strecker degradation, which involves the oxidation of α -amino acids to aldehydes. The amino acids valine, isoleucine, and leucine are oxidized during Strecker degradation to form 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, respectively. These aldehydes can then be reduced to form their respective alcohols, which are less potent. The amino acid methionine undergoes Strecker degradation to form methional, dimethyl sulfide, and dimethyl trisulfide (Griffith and Hammond, 1989; Hofmann et. al., 2000). Phenylacetaldehyde and 2-phenylethanol are formed from the Strecker degradation of phenylalanine (Adamiec et. al., 2001). The heat from steam distillation also catalyzes the formation of β -damascenone and β -ionone from carotenoids (Mendes-Pinto, 2009). In addition to trace odorants formed by heat, many of the odor-active aldehydes in spearmint oil were formed by lipid oxidation. Lipoxygenases catalyze the cleavage of fatty acids in the presence of oxygen. The aldehydes nonanal and (*E,Z*)-2,6-nonadienal are formed from the cleavage of linoleic acid at the C-9 bond, octanal at the C-10 bond, and hexanal at the C-12 bond (Labuza and Dugan, 1971; Vick and Zimmermann, 1979). 1-Octen-3-ol and its reduced form 1-octen-3-one are formed from the cleavage at the C-10 bond to form a C-10 hydroperoxide which is further broken down by a hydroperoxide lyase (Assaf et. al., 1997).

Determination of Odorant Potency

Aroma extract dilution analysis was used to determine the potency of odorants in the spearmint oils. As shown in Table 3.2, (-)-carvone was the most potent odorant in all three spearmint oils. In Native spearmint oil, other potent odorants included eugenol (clove), ethyl-2-methylbutyrate (fruity), β -damascenone (applesauce), (3*E*,5*Z*)-1,3,5-undecatriene (tape), and methional (cooked potato). In Scotch spearmint oil, other potent odorants were eugenol, (3*E*,5*Z*)-1,3,5-undecatriene, β -damascenone, isoeugenol, and an unknown minty odorant ($RI_{WAX} = 1719$). In Macho mint oil, additional potent odorants included eugenol, (3*E*,5*Z*)-1,3,5-undecatriene, ethyl-2-methylbutyrate, and β -damascenone. Important odorants which remain unknown include two minty odorants ($RI_{WAX} = 1425$ and 1719), a bread-like odorant ($RI_{WAX} = 1434$), and an insect repellent-like odorant ($RI_{WAX} = 2366$).

Compound Identification by Gas Chromatography-Mass Spectrometry

While GC-O was used to distinguish the odorants in the spearmint oils and determine their potency, GC-MS analysis was important for the identification of the odor-active compounds. Of the 37 odorants positively identified, only 26 could be detected by GC-MS. Table 3.1 details which compounds were identified by GC-MS. Some compounds, such as dimethyl trisulfide and 3-methyl-2-butene-1-thiol, had high FD factors yet were not detectable by GC-MS. This can be attributed to the low odor thresholds of these compounds. The threshold for dimethyl trisulfide is 0.01 parts per billion, and the threshold for 3-methyl-2-butene-1-thiol is 1.2 parts per trillion (Buttery et. al., 1976; Fritsch and Schieberle, 2005). These trace potent odorants are typically overlooked due to their low concentrations, yet are significant to the overall flavor of spearmint oils.

The results from this study agree with previous literature in that R-(-)-carvone is the most potent odorant in spearmint oil (Jirovetz et. al., 2002; Diaz-Maroto et. al., 2008). However, the next most potent odorants in all three spearmint oils have either never been identified in spearmint oil or have never been shown to contribute to the aroma of spearmint oil. Eugenol, ethyl-2-methyl butyrate, (3*E*,5*Z*)-1,3,5-undecatriene have been identified in spearmint oil, but no studies have indicated their importance to spearmint aroma. Another potent odorant, β -damascenone, has never been identified in spearmint. While GC-O in combination with AEDA is useful in the identification of odorants, a GC-O completely volatilizes compounds and therefore only gives the relative potencies of individual compounds in air. For this reason, GC-O does not account for interactions among the compounds or the matrix in which they exist. To determine the contribution of an odorant within a certain matrix, an odor activity value (OAV) is calculated. The OAV of a compound is found by dividing its odor threshold in a sample matrix by its concentration in the product. These methods and results are detailed in Chapter 4 of this thesis.

3.6 FIGURES AND TABLES

Table 3.1 Aroma-active compounds detected in spearmint oils

No. ^a	Retention Index		Compound	Odor Description	Spearmint Oil ^b	Identification ^c
	RTX- WAX	RTX-5				
1	<900	<700	dimethyl sulfide	corn	N, S, M	RI, O, MS, S
2	<900	<700	2-methyl propanal	malty	N, S, M	RI, O, MS, S
3	921	<700	2-methyl butanal	malty	N, S, M	RI, O, MS, S
4	921	<700	3-methyl butanal	malty	N, S, M	RI, O, MS, S
5	1020	783	methyl-2-methylbutyrate	fruity	N, S, M	RI, O, MS, S
6	1049	897	2,5-diethyltetrahydrofuran	solvent	N, S, M	O, MS
7	1060	851	ethyl-2-methyl butyrate	fruity	N, S, M	RI, O, MS, S
8	1079	861	ethyl-3-methyl butyrate	fruity	N, S, M	RI, O, MS, S
9	-	803	hexanal	grass	N, M	RI, O, MS, S
10	1104	-	1-hexen-3-one	plastic	N, S, M	RI, O, S
11	1115	825	3-methyl-2-butene-1-thiol	skunky	N, S, M	RI, O, S
12	1145	-	unknown	grassy	N, M	-
13	1172	992	myrcene	grassy, pine	N, S, M	RI, O, MS, S
14	1199	1036	limonene	citrus	N, S, M	RI, O, MS, S
15	1211	-	unknown	citrus	S	-
16	1217	1041	1,8-cineole	eucalyptus	N, S, M	RI, O, MS, S
17	1255	-	unknown	pine	N, M	-
18	1295	1005	octanal	citrus	N, M	RI, O, MS, S
19	1301	979	1-octen-3-one	mushroom	N, S, M	RI, O, MS, S
20	1314	-	unknown	musty, bread	N, S, M	-
21	1360	-	unknown	minty	N, S, M	-
22	1370	-	unknown	citrus	N, S, M	-
23	1384	972	dimethyl trisulfide	garlic	N, S, M	RI, O, S
24	1387	1100	nonanal	green, plastic	N, S, M	RI, O, MS, S
25	1395	1172	(3E,5Z)-1,3,5-undecatriene	tape	N, S, M	RI, O, MS, S
26	1425	-	unknown	minty	N, S, M	-
27	1434	-	unknown	bread	N, S, M	-
28	1442	1159	trans-menthone	minty	N, S, M	RI, O, MS, S
29	1453	908	methional	cooked potato	N, S, M	RI, O, S
30	1472	1170	menthone	minty	N, S, M	RI, O, MS, S
31	1505	-	unknown	floral, citrus	N, S, M	-
32	1539	-	unknown	plastic, fresh	N, S, M	-
33	1548	1101	linalool	lavender	N, S, M	RI, O, MS, S
34	1584	1153	(E,Z)-2,6-nonadienal	cucumber	N, S, M	RI, O, S

Table 3.1 continued on next page.

Table 3.1 (continued)

No. ^a	Retention Index		Compound	Odor Description	Spearment Oil ^b	Identification ^c
	RTX- WAX	RTX-5				
35	1597	1187	2-methylisoborneol	earthy	M	RI, O, S
36	1608	-	unknown	fresh	N	-
37	1611	-	unknown	bandaid	S	-
38	1614	1193	trans-dihydrocarvone	minty	N, M	RI, O, MS
39	1625	-	unknown	stale, musty	N, S, M	-
40	1636	1049	phenylacetaldehyde	rose	N, S, M	RI, O, MS, S
41	1649	-	menthol	minty	S	RI, O, MS, S
42	1663	844	isovaleric acid	cheese	N, S, M	RI, O, S
43	1671	-	unknown	bread	N, S, M	-
44	1704	-	unknown	sweet	N, M	-
45	1719	-	unknown	minty	N, S	-
46	1731	1249	R-(-)-carvone [*]	spearment	N, S, M	RI, O, MS, S
47	1746	-	unknown	acidic	S	-
48	1769	-	unknown	cilantro	N, S, M	-
49	1775	1370	<i>E</i> -carvyl acetate	garlic	N, S, M	RI, O, MS, S
50	1801	-	unknown	skunky	M	-
51	1814	-	unknown	grassy	N, S, M	-
52	1816	1349	β -damacenone	applesauce	N, S, M	RI, O, MS, S
53	1835	-	unknown	musty, spices	N, S, M	-
54	1851	-	unknown	sweet, minty	N, S, M	-
55	1878	-	unknown	cilantro	N, M	-
56	1904	1122	2-phenylethanol	rose	N, S, M	RI, O, MS, S
57	1913	-	unknown	floral, sweet	S, M	-
58	1932	1493	β -ionone	floral, sweet	N, S, M	RI, O, MS, S
59	1981	-	caryophyllene oxide	green, plastic	N, S, M	RI, O, MS
60	1995	-	unknown	bread	N, S, M	-
61	2011	-	unknown	fruity, sweet	N, S, M	-
62	2033	-	unknown	rain	N, S	-
63	2066	-	<i>p</i> -cresol	barnyard	N, S, M	RI, O, S
64	2070	-	unknown	minty	S, M	-
65	2107	-	unknown	wet dog	M	-
66	2132	-	unknown	coconut, floral	N, S, M	-
67	2149	1364	eugenol	clove	N, S, M	RI, O, MS, S
68	2167	-	unknown	coconut	N, S	-
69	2193	1320	4-vinyl guaiacol	musty	N, M	RI, O, MS, S
70	2244	-	unknown	fertilizer, grass	N, M	-
71	2282	-	unknown	sweet	N, M	-
72	2317	-	unknown	citrus	M	-

Table 3.1 continued on next page.

Table 3.1 (continued)

No. ^a	Retention Index		Compound	Odor Description	Spearment Oil ^b	Identification ^c
	RTX- WAX	RTX-5				
73	2328	-	isoeugenol	spices	S, M	RI, O
74	2366	-	unknown	insect repellent	N, S, M	-
75	2381	-	unknown	musty, rain	M	-
76	2417	-	unknown	citrus	S	-
77	2443	-	unknown	citrus	M	-
78	2490	-	unknown	citrus	S, M	-
79	2504	-	unknown	citrus	S, M	-
80	2526	1409	vanillin	vanilla	N, S, M	RI, O, S
81	-	<700	2-methyl-1-propanol	malty	N, S, M	RI, O, MS, S
82	-	<700	3-methyl-1-butanol	malty	N, S	RI, O, MS, S
83	-	<700	2-methyl-1-butanol	malty	N, S	RI, O, MS, S
84	-	984	1-octen-3-ol	mushroom	N, S, M	RI, O, S
85	-	1477	γ -decalactone	peach	N, S	RI, O, MS, S

^aNumbers correspond to those in Tables 3.1 - 3.3. ^bSpearment oil in which the odorant was detected: Native Spearment (N), Scotch spearment (S), Macho mint (M). ^cMethod of identification: retention index (RI), odor quality (O), mass spectra (MS), reference standard compound (S). * Presumed enantiomer based on literature.

Table 3.2 Flavor dilution factors of compounds detected in spearmint oils

No. ^a	Retention Index		Compound	Odor Description	FD Factor ^b		
	RTX-WAX	RTX-5			Native	Scotch	Macho
3	921	<700	2-methyl butanal	malty	1024	2048	32
4	921	<700	3-methyl butanal	malty	1024	2048	32
5	1020	783	methyl-2-methylbutyrate	fruity	512	2048	256
6	1049	897	2,5-diethyltetrahydrofuran	solvent	1024	256	256
7	1060	851	ethyl-2-methyl butyrate	fruity	32768	2048	16384
8	1079	861	ethyl-3-methyl butyrate	fruity	64	32	128
10	1104	-	1-hexen-3-one	plastic	8	128	64
11	1115	825	3-methyl-2-butene-1-thiol	skunky	64	2048	2048
12	1145	-	unknown	grassy	8	-	16
13	1172	992	myrcene	grassy, pine	1024	512	2048
14	1199	1036	limonene	citrus	1024	128	1024
15	1211	-	unknown	citrus	-	256	-
17	1255	-	unknown	pine	8	-	2048
18	1295	1005	octanal	citrus	8	-	64
19	1301	979	1-octen-3-one	mushroom	256	512	128
20	1314	-	unknown	musty, bread	64	512	128
21	1360	-	unknown	minty	32	64	64
22	1370	-	unknown	citrus	32	32	64
23	1384	972	dimethyl trisulfide	garlic	32	512	2048
24	1387	1100	nonanal	green	16	-	-
25	1395	1172	(3E,5Z)-1,3,5-undecatriene	tape	4096	16384	32768
26	1425	-	unknown	minty	128	1024	256
27	1434	-	unknown	bread	1024	512	256
28	1442	1159	trans-menthone	minty	1024	512	512
29	1453	908	methional	cooked potato	4096	512	1024
30	1472	1170	menthone	minty	64	256	32
31	1505	-	unknown	floral, citrus	32	32	256
32	1539	-	unknown	plastic, fresh	128	128	128
33	1548	1101	linalool	lavender	1024	512	2048
34	1584	1153	(E,Z)-2,6-nonadienal	cucumber	2048	1024	2048
35	1597	1187	2-methylisoborneol	earthy	-	-	256
36	1608	-	unknown	fresh	64	-	-
37	1611	-	unknown	bandaid	-	32	-
38	1614	1193	trans-dihydrocarvone	minty	16	-	16
39	1625	-	unknown	stale, musty	64	64	64
40	1636	1049	phenylacetaldehyde	rose	1024	128	1024
41	1649	-	menthol	minty	-	32	-

Table 3.2 continued on next page.

Table 3.2 (continued)

No. ^a	Retention Index		Compound	Odor Description	FD Factor ^b		
	RTX-WAX	RTX-5			Native	Scotch	Macho
43	1671	-	unknown	bread	128	512	256
44	1704	-	unknown	sweet	8	-	16
45	1719	-	unknown	minty	2048	8192	-
46	1731	1249	R-(-)-carvone*	spearmint	>131072	>524288	>262144
47	1746	-	unknown	acidic	-	256	-
48	1769	-	unknown	cilantro	8	128	16
49	1775	1370	E-carvyl acetate	garlic	4096	1024	512
50	1801	-	unknown	skunky	-	-	16
51	1814	-	unknown	grassy	128	64	16
52	1816	1349	β-damascenone	applesauce	8192	4096	4096
53	1835	-	unknown	musty, spices	16	512	16
54	1851	-	unknown	sweet, minty	16	32	16
55	1878	-	unknown	cilantro	8	-	16
56	1904	1122	2-phenylethanol	rose	2048	512	128
57	1913	-	unknown	floral, sweet	-	32	32
58	1932	1493	β-ionone	floral, sweet	1024	256	256
59	1981	-	caryophyllene oxide	green, plastic	8	32	16
60	1995	-	unknown	bread	64	128	32
61	2011	-	unknown	fruity, sweet	8	32	16
62	2033	-	unknown	rain	64	32	-
63	2066	-	p-cresol	barnyard	64	128	128
64	2070	-	unknown	minty	-	32	16
65	2107	-	unknown	wet dog	-	-	32
67	2149	1364	eugenol	clove	65536	65536	131072
68	2167	-	unknown	coconut	64	32	-
69	2193	1320	4-vinyl guaiacol	musty	64	-	32
70	2244	-	unknown	fertilizer, grass	8	-	128
71	2282	-	unknown	sweet	8	-	16
72	2317	-	unknown	citrus	-	-	16
73	2328	-	isoeugenol	spices	-	4096	16
74	2366	-	unknown	insect repellent	2048	1024	256
75	2381	-	unknown	musty, rain	-	-	32
76	2417	-	unknown	citrus	-	64	-
77	2443	-	unknown	citrus	-	-	32
78	2490	-	unknown	citrus	-	32	32
79	2504	-	unknown	citrus	-	32	16
80	2526	1409	vanillin	vanilla	64	64	64

^aNumbers correspond to those in Tables 3.1 - 3.3. ^bFlavor dilution factors were determined using a RTX-WAX column. *Presumed enantiomer based on literature.

Table 3.3 Odorants previously identified in spearmint oils

No. ^a	Compound	Odor Description	Previously Identified in Spearmint	Previously Identified as an Odorant in Spearmint
1	dimethyl sulfide	corn	Y ⁱ	Y ⁱ
2	2-methyl propanal	malty	Y ⁱ	Y ⁱ
3	2-methyl butanal	malty	Y ⁱ	Y ⁱ
4	3-methyl butanal	malty	Y ⁱ	Y ⁱ
5	methyl-2-methylbutyrate	fruity	Y ⁱ	Y ⁱ
7	ethyl-2-methylbutyrate	fruity	Y ^b	N
8	ethyl-3-methylbutyrate	fruity	Y ^c	Y ^c
9	hexanal	grass		
10	1-hexen-3-one	plastic	N	N
11	3-methyl-2-butene-1-thiol	skunky	N	N
13	myrcene	grassy, pine	Y ^d	Y ^d
14	limonene	citrus	Y ^d	Y ^d
16	1,8-cineole	eucalyptus	Y ^c	Y ^c
18	octanal	citrus	Y ^c	Y ^c
19	1-octen-3-one	mushroom	Y ^c	Y ^c
23	dimethyl trisulfide	garlic	N	N
24	nonanal	green, plastic	Y ^e	N
25	(3 <i>E</i> ,5 <i>Z</i>)-1,3,5-undecatriene	tape	Y ^f	N
28	trans-menthone	minty	Y ^b	N
29	methional	cooked potato	Y ^c	Y ^c
30	menthone	minty	Y ^h	Y ^h
33	linalool	lavender	Y ^c	Y ^c
34	(<i>E</i> , <i>Z</i>)-2,6-nonadienal	cucumber	N	N
35	2-methylisoborneol	earthy	N	N
38	trans-dihydrocarvone	minty	Y ^d	Y ^d
40	phenylacetaldehyde	rose	Y ^b	N
41	menthol	minty	Y ^d	Y ^d
42	isovaleric acid	cheese	Y ^c	Y ^c
46	R-(-)-carvone*	spearmint	Y ^c	Y ^c
49	<i>E</i> -carvyl acetate	garlic	Y ^d	Y ^d
52	β-damascenone	applesauce	N	N
56	2-phenylethanol	rose	Y ^b	N
58	β-ionone	floral, sweet	Y ^b	N
63	<i>p</i> -cresol	barnyard	Y ^g	N
67	eugenol	clove	Y ^g	N
69	4-vinyl guaiacol	musty	N	N
80	vanillin	vanilla	Y ^g	N
81	1-octen-3-ol	mushroom	Y ^c	Y ^c
82	γ-decalactone	peach	N	N

^aNumbers correspond to those in Tables 3.1 - 3.3. ^bLawrence, 2007. ^cDiaz-Maroto et. al., 2008.

^dJirovetz et. al. 2002. ^eKokkini and Vokou, 1989. ^fMookherjee et. al., 1990. ^gTsuneya et. al., 1998. ^hColeman et. al., 2002. ⁱColeman et. al., 2004. *Presumed enantiomer based on literature.

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CHAPTER FOUR

QUANTITATION OF POTENT ODORANTS

4.1 ABSTRACT

Forty-six compounds in Native spearmint, Scotch spearmint, and Macho mint oils were quantified using various methods. Nineteen high abundance compounds were quantified by gas chromatography with a flame ionization detector (GC-FID), 20 were quantified by stable isotope dilution analysis (SIDA), and 14 were quantified by gas chromatography-olfactometry (GC-O) dilution analysis. Seven of the compounds quantified by GC-O dilution analysis were also quantified by SIDA. The concentration results were used to calculate the odor activity value (OAV) of each compound by dividing the concentration by the odor detection threshold of the compound in water. Among the compounds quantified, those with the highest OAVs were R-(-)-carvone, 1,8-cineole, (*E,Z*)-2,6-nonadienal, β -damascenone, and (*3E,5Z*)-1,3,5-undecatriene.

4.2 KEYWORDS

Spearmint, gas chromatography, gas chromatography-mass spectroscopy, gas chromatography-olfactometry, stable isotope dilution analysis

4.3 INTRODUCTION

Spearmint has been popular in the United States since the late 1700's, and the industry has grown to produce 2.93 million pounds of spearmint oil in 2013. While spearmint oil is commonly used in confectionery and oral healthcare products, there have been few studies on the flavor chemistry of spearmint. In the previous study of this thesis, 65 odorants were detected in Native spearmint, 61 in Scotch spearmint, and 59 in Macho mint using gas chromatography-olfactometry (GC-O) in combination with aroma extract dilution analysis (AEDA) (Chapter Three). Some of the most potent compounds in the spearmint oils were R-

(-)-carvone (spearmint), eugenol (clove), ethyl-2-methyl butyrate (fruity), β -damascenone (applesauce), and (3*E*,5*Z*)-1,3,5-undecatriene (tape). Other potent odorants include 3-methyl-2-butene-1-thiol (skunk), methional (cooked potato), linalool (lavender), and phenylacetaldehyde (rose).

GC-O completely volatilizes compounds, so AEDA results only indicate the potency of the individual compounds in air. For this reason, GC-O does not account for interactions among the compounds or the matrix in which they exist. To determine the contribution of an odorant within a certain matrix, an odor activity value (OAV) is calculated. The OAV of a compound is found by dividing its odor threshold in a sample matrix by its concentration in the product (Grosch, 1993).

To find the concentrations of odorants in the spearmint oils, several quantitation methods were implemented. Stable isotope dilution analysis (SIDA) involves using either a deuterium or carbon-13 labeled isotope of the target analyte as an internal standard to determine the concentration of that compound. SIDA gives precise and accurate quantitation results since a labeled isotope is nearly identical to the target analyte, and therefore the isotope is a perfect internal standard. However, for trace potent odorants that are not detectable by GC-MS, SIDA is not a viable option. GC-O dilution analysis can be used to determine the concentrations of trace compounds with low odor thresholds by comparing the flavor dilution (FD) factor of a compound with a known concentration to the FD factor of that compound in a sample. Additionally, SIDA is not a practical method to determine the concentration of compounds in high abundance. Quantitation using a GC-FID can be used to quantify high abundance compounds by comparing their peak areas with that of an internal standard.

4.4 MATERIALS AND METHODS

Materials

Farwest Native spearmint (crop year 2011, manufactured May 3, 2013), Farwest Scotch spearmint (crop year 2012, manufactured September 3, 2012), and Macho mint oil were obtained from the Wm. Wrigley Jr. Company (Chicago, IL).

Chemicals

n-Alkane standards, 2-methyl-3-heptanone, 6-undecanone, tert-butyl benzene, 1-hexadecene, and ethyl acetate (99.5%) were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO). Methanol (99.9%), methylene chloride (99.9%), diethyl ether (anhydrous, 99.9%), and pentane (99.4%) were acquired from Thermo Fisher Scientific Inc. (Waltham, MA). Liquid nitrogen, ultra-high purity (UHP) nitrogen gas, UHP helium, and UHP hydrogen were purchased from S.J. Smith Co. (Davenport, IA).

Standard Compounds. (*E,Z*)-2,6-nonadienal, phenylacetaldehyde, isovaleric acid, eugenol, vanillin, linalool, β -ionone, β -damascenone, *p*-cresol, hexanal, octanal, nonanal, dimethyl sulfide, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO). 1,3,5-Undecatriene was purchased from Bedoukian Research, Inc. (Danbury, CT). 1-Hexen-3-one and 1-octen-3-one were obtained from Alfa Aesar (Ward Hill, MA).

Isotope Standard Compounds. The following isotopically-labeled standards were obtained from the commercial sources listed in parentheses: [2H_6]-dimethyl sulfide (Sigma-Aldrich; St. Louis, MO); [2H_2]-3-methylbutanal (CDN, Quebec, Canada).

Syntheses

The following compounds were synthesized according to the procedures reported in the literature given in parentheses: [2H_4]-octanal, [2H_4]-nonanal, [2H_3]-eugenol (Lorjaroenphon, 2012), [2H_4]- β -damascenone and [2H_3]- β -ionone (Kotseridis et. al., 1998), 2-[$^{13}C_2$]-phenylethanol and [$^{13}C_2$]-phenylacetaldehyde (Schuh and Schieberle, 2006), [2H_4]- (3E,5Z)-1,3,5-undecatriene (Steinhaus and Schieberle, 2000; Schieberle and Steinhaus, 2001), [2H_2]-3-methyl-1-butanol (Steinhaus and Schieberle, 2005), [2H_2]-2-methyl-1-propanol and [2H_2]-ethyl-3-methyl butyrate (Lahne, 2010), [2H_2]-linalool (Steinhaus et. al., 2003).

2-methyl-[3,4- 2H_2]-butan-1-ol was synthesized according to the method previously described for the synthesis of 3-methyl-[3,4- 2H_2]-butan-1-ol with slight modification (Steinhaus and Schieberle, 2005). Chlorotri(triphenylphosphine)rhodium(I) (Wilkinson's catalyst, 0.15 g)(Aldrich), 2-methyl-3-buten-1-ol (0.950 g, 11.0 mmol)(Aldrich) were placed in a pressure reactor equipped with stirring bar and rubber septum. The reactor was flushed for 5 min with deuterium gas (40 psi; UHP grade 99.995%; isotopic enrichment 99.7%; Matheson Tri-Gas, Parsippany, NJ, USA) using a needle, which was placed below the solution. The spent catalyst was removed by centrifugation after the reaction was complete. 2-Methyl-[3,4- 2H_2]-butan-1-ol was obtained after purification by vacuum distillation.

Yield of 2-methyl-[3,4- 2H_2]-butan-1-ol: 0.470 g (49.5 %). MS-EI, m/z (intensity in %): 59 (100), 58 (83), 72 (59), 57 (58), 42 (56), 43 (41), 71 (39), 41 (30), 56 (22), 40 (18), 90 (1, M^+).

3-Methyl-2-butene-1-thiol was synthesized as previously reported for butylthiol, with 3,3-dimethylallyl bromide (Sigma Aldrich, St. Louis, MO) being substituted for *n*-butyl bromide

(Ishikawa et. al., 1984). MS-EI, m/z (intensity in %): 41(100), 69 (58), 102 (33, M^+), 68 (28), 39 (22), 53 (16), 67 (10), 45 (8), 59 (5), 47 (5).

Quantitation by Gas Chromatography

Spearmint oil (50 mg) was spiked with solutions of the internal standards *tert*-butyl benzene, 2-methyl-3-heptanone, and 6-undecanone. The spearmint oils were then extracted using a silica gel column (1 g) chromatography and pentane (10 mL) as a mobile phase followed by 90:10 pentane:ether (10 mL). The fractions were concentrated with a nitrogen gas stream to 0.2 mL and analyzed on an Agilent 6890 GC-FID (Agilent Technologies, Inc., Santa Clara, CA) equipped with a nonpolar DB-5 column (50 m x 0.32 mm x 1 μ m; J&W Scientific, Folsom, CA). The samples were injected in split mode (20:1) with an inlet temperature of 280 °C. The oven temperature was held at 50 °C for 5 minutes, ramped to 225 °C at 4 °C/min, and held at 225 °C for 30 minutes. The peak areas of the analytes were compared to the peak area of the internal standard in each extraction. *tert*-Butyl benzene was the internal standard in the nonpolar (pentane) fraction. 2-Methyl-3-heptanoate and 6-undecanone were the internal standards for the polar (90:10 pentane:ether) fraction.

Stable Isotope Dilution Analysis

The deuterium or carbon-13 labeled isotopes of the target analytes were prepared in solutions of ether, ethyl acetate, dichloromethane, or pentane to dilute the compounds to working concentrations. The isotope solutions were spiked into each of the three spearmint oils. The concentrations of the isotopes spiked into the spearmint oils are listed in Appendix A. Three replications of each spearmint oil were analyzed. The spiked spearmint oils were either run neat or diluted in solvents, depending on the analyte. The samples were analyzed using either a Stabliwax® column (30 m x 0.25 mm ID, 0.25 μ m; Restek Corporation, Bellefonte, PA) or a

SAC-5 column (30 m x 0.25 mm ID, 0.25 μ m; Sigma-Aldrich Co. LLC., St. Louis, MO). Except where noted, the GC-MS parameters were as follows. The GC-MS system consisted of a 6890 GC-HP 5973N mass selective detector (Agilent Technologies, Inc.). The inlet temperature was cryo-cooled to -50 °C to reduce the loss of highly volatile compounds and prevent the formation of artifacts in the inlet. The oven temperature was held at 40 °C for 5 minutes, ramped to 225 °C at 4 °C/min, and held at 225 °C for 30 minutes. Helium was used as the carrier gas with a flow rate of 0.8 mL/min. The mass scan range for the mass spectrometer was 35 to 300 m/z . The ionization energy for the mass spectrometer was 70 eV. The samples were analyzed using the total ion current (TIC) mode on the mass spectrophotometer.

Eugenol. Spearmint oil (20 mg) was spiked with a solution of [2H_3]-eugenol. The samples were injected neat, and analyzed using a GC-MS with a Stabilwax column.

β -*Damascenone* and β -*Ionone*. Spearmint oil (250 mg) was spiked with solutions of [2H_4]- β -damascenone and [2H_3]- β -ionone. The samples were injected neat, and analyzed using a GC-MS with a Stabilwax column.

Linalool. Spearmint oil (50 mg) was spiked with a solution of [2H_2]-linalool and diluted with pentane. The analytes were extracted using a silica gel column (1 g) and 95:5 pentane:ether (10 mL) followed by 90:10 pentane:ether (10 mL). The 90:10 pentane:ether extract was retained and concentrated using a nitrogen gas stream. The extracts were analyzed using a GC-MS with a SAC-5 column.

Strecker Aldehydes, Strecker Alcohols, and Dimethyl Sulfide. Spearmint oil (100 mg) was spiked with solutions of [2H_6]-dimethyl sulfide, [2H_2]-3-methylbutanal, [2H_2]-3-methyl-1-butanol, [2H_2]-2-methyl-1-butanol, and [2H_2]-2-methyl-1-propanol. [2H_2]-3-methylbutanal was used to

quantify 3-methylbutanal, 2-methylbutanal, and 2-methylpropanal. The other labeled isotopes were used only to quantify the corresponding unlabeled compound. The spiked spearmint oil (2 μ L) was analyzed using a hot split (260 °C) injection and a SAC-5 column (30 m x 0.25 mm ID, 0.25 μ m; Sigma-Aldrich Co. LLC., St. Louis, MO). The oven was cyro-cooled to 20 °C and held for 5 minutes, ramped to 225 °C at 15 °C/min, and held at 225 °C for 30 minutes.

Hexanal, Octanal, and Nonanal. Spearmint oil (50 mg) was spiked with solutions of [2H_4]-octanal and [2H_4]-nonanal. [2H_4]-octanal was used for the quantitation of hexanal and octanal. The spiked spearmint oil was diluted with pentane and extracted using a silica gel column (1 g). The spearmint oil was eluted with pentane (10 mL) followed by 95:5 pentane:ether (10 mL). The 95:5 pentane:ether extract was retained and concentrated with a nitrogen gas stream. The extracts were analyzed using a GC-MS with a Stabilwax column.

(3E,5Z)-1,3,5-Undecatriene, Phenylacetaldehyde, Phenylethanol. Spearmint oil (50 mg) was spiked with solutions of [2H_4]-*(3E,5Z)*-1,3,5-undecatriene, [$^{13}C_2$]-phenylacetaldehyde, and [$^{13}C_2$]-phenylethanol and diluted with pentane. The mixture was extracted using a silica gel column (1 g), and eluting with pentane (10 mL), 95:5 pentane:ether (10 mL), 90:10 pentane:ether (10 mL), and 80:20 pentane:ether (10 mL). The pentane (containing [2H_4]-*(3E,5Z)*-1,3,5-undecatriene), 95:5 pentane:ether (containing [$^{13}C_2$]-phenylacetaldehyde), and 80:20 pentane:ether (containing [$^{13}C_2$]-phenylethanol) extracts were concentrated with a nitrogen gas stream. The extracts were analyzed using a GC-MS with a Stabilwax column. Phenylethanol and *(3E,5Z)*-1,3,5-undecatriene were analyzed using the total ion current (TIC) mode on the mass spectrophotometer, while phenylacetaldehyde was analysis using selected ion monitoring (SIM).

Methyl-2-methyl butyrate, ethyl-2-methyl butyrate, ethyl-3-methyl butyrate. Spearmint oil (50 mg) was spiked with a solution of [$^2\text{H}_2$]-ethyl-3-methyl butyrate. The samples were injected neat, and analyzed using a GC-MS with a Stabilwax column. The selected ions were monitored using SIM mode.

Calibration of Isotopes

To accurately calculate the concentration of the analytes in the spearmint oils, it was necessary to determine the response factors for the labeled isotopes. The response factor for each isotope to its target analyte was computed by making a calibration curve. For each calibration curve, a solution containing the same concentration of isotope as was used to spike the spearmint oil was made in either ethyl acetate, ether, methanol, or dichloromethane. However, for the highly volatile Strecker aldehydes, Strecker alcohols, and dimethyl sulfide, a spearmint matrix of R-(-)-carvone (86%) and limonene (14%) was used instead of a solvent. Each solution was spiked with increasing amounts of the unlabeled compound, resulting in five different concentrations of the unlabeled standard. After every spike, the solution was analyzed using GC-MS. The peak areas of the selected ion for the analyte and corresponding labeled isotope were integrated using MSD ChemStation Enhanced Data Analysis Software (Agilent Technologies, Inc., Santa Clara, CA). To generate the calibration curves, the mass ratios of the analyte to the isotope were plotted against the ratio of the peak area of the selected ion for the unlabeled analyte and the labeled isotope on a scatter plot. The response factor of the isotope was found by taking the reciprocal of the slope from the linear trendline.

Calculation of Concentration

To determine the concentration of the analytes, the peak areas of the selected ion for the analyte and corresponding labeled isotope were integrated using MSD ChemStation Enhanced

Data Analysis Software (Agilent Technologies, Inc., Santa Clara, CA). The following equation was used to calculate the concentration of the analyte in spearmint oil:

$$\frac{Mass_{analyte}}{Mass_{internal\ standard}} = \frac{Area_{analyte}}{Area_{internal\ standard}} (R_f)$$

Gas Chromatography – Olfactometry Dilution Analysis

Solutions of each compound were made in ethyl acetate (10 mL). Two solutions were made by combining several of the compound solutions (1 mL each) and diluting to 10 mL. The first solution contained 1-hexen-3-one, octanal, 1-octen-3-one, (3*E*,5*Z*)-1,3,5-undecatriene, phenylacetaldehyde, and isovaleric acid. The second solution contained (*E*,*Z*)-2,6-nonadienal, β -damacenone, phenylethanol, β -ionone, *p*-cresol, eugenol, and vanillin. A separate solution was made by dissolving 3-methyl-2-butene-1-thiol in ether. From the solutions, 1:2 serial dilution series were made. The dilutions were analyzed by two people using GC-O with an RTX®-WAX column (15 m x 0.53 mm ID x 1 μ m; Restek Corporation, Bellefonte, PA) to determine the threshold of each compound.

The concentration of each of the compounds was determined first by calculating the concentration of the compounds in the solutions based on their purity and initial dilution. The concentration in the solution was then divided by the dilution threshold of the compound and multiplied by the dilution threshold in the mint oil to give the concentration of each compound in the mint oil.

$$\begin{aligned} &\text{Concentration in Solution (ppm)} \\ &= (\text{Purity})(\text{Weight in mg})(\text{Dilution in final solution of 10 mL})(100) \end{aligned}$$

$$\begin{aligned} &\text{Concentration in Mint Oil (ppm)} \\ &= (\text{Concentration in solution})\left(\frac{1}{\text{dilution threshold}}\right)(\text{dilution threshold in mint oil}) \end{aligned}$$

4.5 RESULTS AND DISCUSSION

Overall, 46 compounds were quantified. Of these compounds, 19 were quantified by GC-FID, 13 were quantified solely by SIDA, seven were quantified solely by GC-O dilution analysis, and seven were quantified by both GC-O dilution analysis and SIDA.

There were 19 compounds in high abundance that were quantified using a GC-FID. Flash chromatography was used to separate the spearmint oils into nonpolar (pentane) and polar (90:10 pentane:ether) fractions. Twelve compounds were quantified from the nonpolar fraction, and seven compounds were quantified from the polar fraction. The compounds were quantified by comparing their peak areas to the peak areas of internal standards added to the spearmint oil. By using one internal standard to quantify high abundance compounds, it is more time and cost effective than quantifying using SIDA. Furthermore, since the peak areas of the high abundance compounds are so large, an internal standard of similar size and polarity to the analytes can be used to accurately quantify the compounds. While most of the high abundance compounds are not odor-important, their concentrations are important to the overall understanding of spearmint oil composition.

Of the 46 compounds quantified, 20 were quantified by SIDA. A deuterium or carbon-13 labeled isotope of a target analyte is the perfect internal standard, because the isotope and unlabeled compound are nearly identical with the exception of their mass spectra. Due to the similarity between a labeled isotope and an unlabeled analyte, quantitations performed by SIDA are highly precise and accurate. Though, for some of the compounds, an isotope similar in structure was used rather than the labeled isotope of that analyte; [$^2\text{H}_2$]-3-methylbutanal was used as the internal standard for 2-methylbutanal and 2-methylpropanal and [$^2\text{H}_4$]-octanal was used as the internal standard for hexanal.

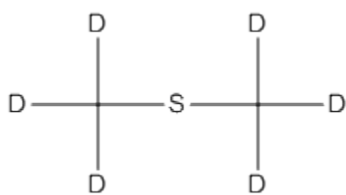
GC-O dilution analysis was used to quantify 14 compounds. Of these compounds, seven were also quantified by SIDA. It is expected that the concentrations found by GC-O dilution analysis would be within a 2-fold range of the actual value, since the compounds were analyzed from a 1:2 serial dilution series. Some of the concentrations of compounds quantified by GC-O dilution analysis (β -ionone and octanal) are within a 2-fold range of the concentrations determined by SIDA. Others are within a 4-fold range. In comparison, GC-O dilution analysis is not as accurate and precise as SIDA. While the results do not have very high accuracy, GC-O dilution analysis allows for the quantitation of trace potent odorants that cannot be easily quantified on a GC-MS because of their low concentrations. Additionally, GC-O dilution analysis does not need to be performed by multiple people for one sample, because a person's detection threshold for a compound will not change.

From the concentrations found using quantitation by GC-FID, SIDA, and GC-O dilution analysis, the odor activity values (OAVs) were calculated. For all the spearmint oils, R-(-)-carvone (spearmint) had the highest OAV. Other potent compounds in Native spearmint oil were 1,8-cineole (eucalyptus), (*E,Z*)-2,6-nonadienal (cucumber), limonene (citrus), and β -damascenone (applesauce). In Scotch spearmint oil, other compounds with high OAVs were β -damascenone, 1,8-cineole, (*E,Z*)-2,6-nonadienal, and 3-methylbutanal (malty). In Macho mint oil, other odor important compounds were (*3E,5Z*)-1,3,5-undecatriene (tape), (*E,Z*)-2,6-nonadienal, 1,8-cineole, and β -ionone (floral).

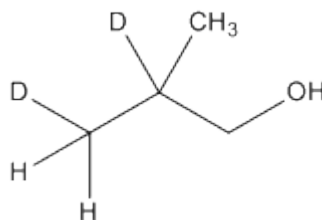
The results from this study agree with previous literature in that R-(-)-carvone is the most important odorant in spearmint oil. Previous literature has also shown that 1,8-cineole and limonene are highly important to the aroma of Native spearmint oil (Jirovetz et. al., 2002; Diaz-Maroto et. al., 2008). The OAV's calculated for Native spearmint also indicate that 1,8-cineole

and limonene are among the most potent compounds, with the second and fourth highest OAV's, respectively. While several of the potent odorants in this study have previously been indicated as important to the flavor of spearmint, many have been overlooked in earlier studies. (*E,Z*)-2,6-nonadienal and β -damascenone were among the highest OAV's for all three spearmint oils, yet they have never been reported in spearmint oil. Additionally, (*3E,5Z*)-1,3,5-undecatriene had the second highest OAV in Macho mint, though it has not been previously reported as contributing to the aroma of spearmint oil.

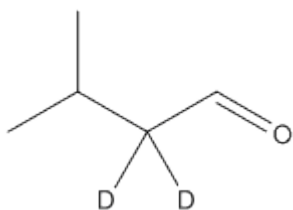
4.6 FIGURES AND TABLES



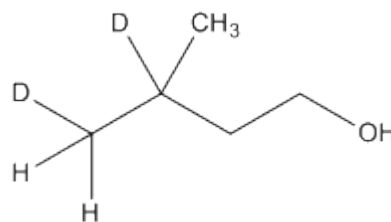
I - 1 [2H_6]-dimethyl sulfide



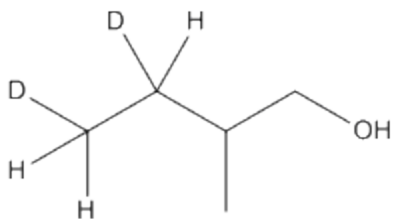
I - 81 [2H_2]-2-methyl-1-propanol



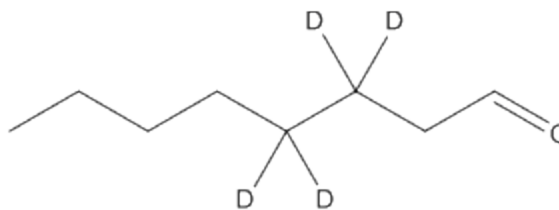
I - 4 [2H_2]-3-methylbutanal



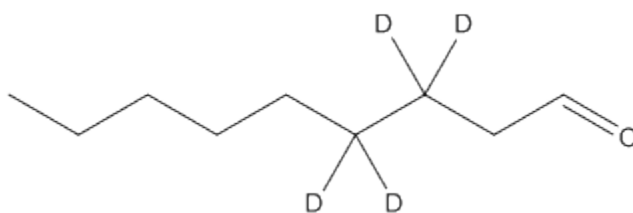
I-82 [2H_2]-3-methyl-1-butanol



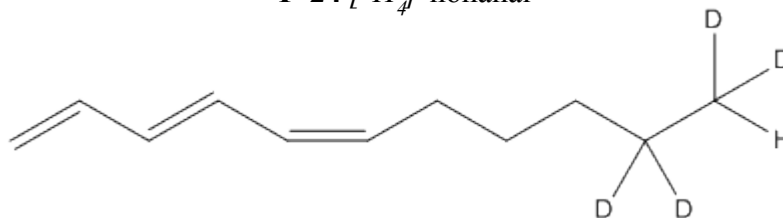
I-83 [2H_2]-2-methyl-1-butanol



I - 18 [2H_4]-octanal



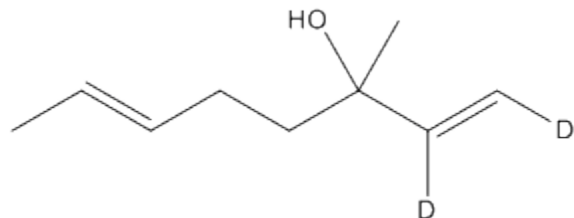
I -24 [2H_4]-nonanal



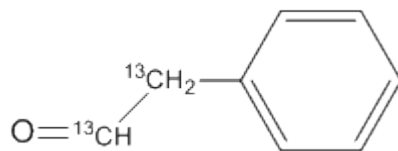
I - 25 [2H_4]-(*3E,5Z*)-1,3,5-undecatriene

Figure 4.1 Chemical structures of isotope standards

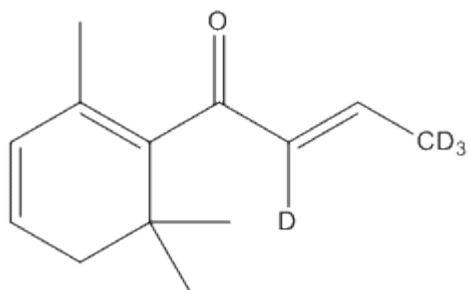
Figure 4.1 (continued)



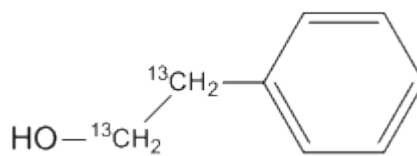
I - 33 [2H_2]-linalool



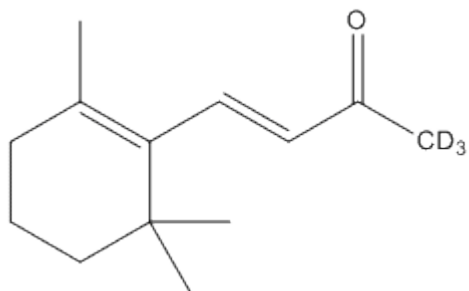
I - 40 [$^{13}C_2$]-phenylacetaldehyde



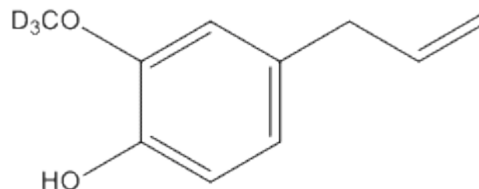
I - 52 [2H_4]- β -damascenone



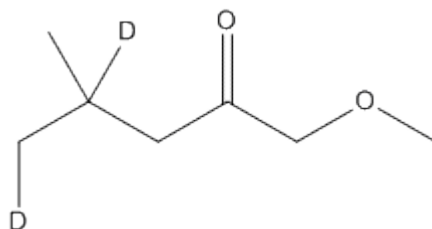
I - 56 [$^{13}C_2$]-phenylethanol



I - 58 [2H_3]- β -ionone



I - 67 [2H_3]-eugenol



I - 8 [2H_3]-ethyl-3-methyl
butyrate

Table 4.1 Analytes, labeled isotopes, selection ions, and response factors used for SIDA

Analyte ^a		Labeled Isotope ^b		Selection Ion (<i>m/z</i>)		R _f ^a
				Analyte	Labeled Isotope	
eugenol	(67)	[² H ₃]-eugenol	(I-67)	164	167	1.01
linalool	(33)	[² H ₂]-linalool	(I-33)	121	123	0.99
hexanal	(9)	[² H ₄]-octanal	(I-18)	56	104	0.080
octanal	(18)	[² H ₄]-octanal	(I-18)	102	104	0.74
nonanal	(24)	[² H ₄]-nonanal	(I-24)	114	116	0.92
dimethyl sulfide	(1)	[² H ₆]-dimethyl sulfide	(I-1)	62	68	0.32
2-methylpropanal	(2)	[² H ₂]-3-methylbutanal	(I-4)	72	88	0.070
3-methylbutanal	(4)	[² H ₂]-3-methylbutanal	(I-4)	86	88	0.50
2-methylbutanal	(3)	[² H ₂]-3-methylbutanal	(I-4)	86	88	0.23
2-methyl-1-propanol	(81)	[² H ₂]-2-methyl-1-propanol	(I-81)	74	76	0.49
3-methyl-1-butanol	(82)	[² H ₂]-3-methyl-1-butanol	(I-82)	70	72	0.25
2-methyl-1-butanol	(83)	[² H ₂]-2-methyl-1-butanol	(I-83)	70	72	0.47
β-damascenone	(52)	[² H ₄]-β-damascenone	(I-52)	190	194	2.50
β-ionone	(58)	[² H ₃]-β-ionone	(I-58)	177	180	2.16
(3 <i>E</i> ,5 <i>Z</i>)-1,3,5-undecatriene	(25)	[² H ₄]-(<i>3E,5Z</i>)-1,3,5-undecatriene	(I-25)	150	154	1.36
phenylacetaldehyde	(40)	[¹³ C ₂]-phenylacetaldehyde	(I-40)	120	122	5.24
2-phenylethanol	(56)	[¹³ C ₂]-2-phenylethanol	(I-56)	122	124	2.57
methyl-2-methyl butyrate	(5)	[² H ₃]-ethyl-3-methyl butyrate	(I-8)	101	117	0.42
ethyl-2-methyl butyrate	(7)	[² H ₃]-ethyl-3-methyl butyrate	(I-8)	115	117	1.00
ethyl-3-methyl butyrate	(8)	[² H ₃]-ethyl-3-methyl butyrate	(I-8)	115	117	0.82

^aNumbers refer to those in Tables 4.1-4.5 and Chapter 3. ^bNumbers refer to those in Figure 4.1.

^cResponse factor of the analyte to its labeled isotope.

Table 4.2 Concentrations of compounds quantified by SIDA

No. ^a	Compound	Concentration (µg/g; ppm) ^b								
		Native			Scotch			Macho		
67	eugenol	475	±	35	285	±	8	320	±	13
33	linalool	733	±	42	649	±	21	507	±	42
9	hexanal	26.3	±	4.7	29.1	±	8	23.9	±	4.5
18	octanal	226	±	18	115	±	10	288	±	19
24	nonanal	71.2	±	12	181	±	20	206	±	11
1	dimethyl sulfide	71.2	±	1.1	27.3	±	3.4	10.9	±	0.8
2	2-methylpropanal	534	±	53	315	±	22	80.7	±	9
4	3-methylbutanal	921	±	113	610	±	41	154	±	11
3	2-methylbutanal	558	±	31	363	±	28	102	±	4.1
81	2-methyl-1-propanol	104	±	0.8	44.8	±	3.1	12.7	±	1.7
82	3-methyl-1-butanol	221	±	8	116	±	3.3	-		
83	2-methyl-1-butanol	188	±	13	88.1	±	2.2	-		
52	β-damascenone	14.7	±	2.7	26.3	±	2.5	7.25	±	0.59
58	β-ionone	10.0	±	5	17.4	±	0.9	37.1	±	2.8
25	(3 <i>E</i> ,5 <i>Z</i>)-1,3,5-undecatriene	122	±	6	55.8	±	6	245	±	4.1
40	phenylacetaldehyde	219	±	27	147	±	12	244	±	27
56	2-phenylethanol	224	±	11	307	±	2.5	152	±	3.6
5	methyl-2-methyl butyrate	356	±	55	201	±	23	172	±	25
7	ethyl-2-methyl butyrate	216	±	30	125	±	11	177	±	17
8	ethyl-3-methyl butyrate	14.7	±	1.8	6.57	±	0.70	13.6	±	1.2

^aNumbers refer to those in Tables 4.1-4.5 and Chapter 3. ^bAverage ± standard deviation (n=3).

Table 4.3 Concentrations of compounds quantified by GC-FID

Compound	Concentration ($\mu\text{g/g}$; ppm) ^a								
	Native			Scotch			Macho		
R-(-)-carvone*	752,000	$\pm$	17,200	767,000	$\pm$	10,700	670,000	$\pm$	13,400
limonene	97,100	$\pm$	2,370	131,000	$\pm$	3,330	117,000	$\pm$	4,560
myrcene	21,300	$\pm$	675	8,960	$\pm$	196	23,000	$\pm$	819
1,8-cineole	19,100	$\pm$	357	12,400	$\pm$	398	12,500	$\pm$	230
4-terpineol	12,700	$\pm$	1,430	3,200	$\pm$	329	1,690	$\pm$	133
<i>E</i> -carvyl acetate	4,700	$\pm$	979	1,460	$\pm$	291	6,920	$\pm$	1,140
γ -terpinene	4,530	$\pm$	76	1,210	$\pm$	33	833	$\pm$	35
α -pinene	4,180	$\pm$	290	5,250	$\pm$	133	6,880	$\pm$	153
dihydrocarvyl acetate	4,040	$\pm$	711	972	$\pm$	152	7,540	$\pm$	1,130
3-octyl acetate	3,430	$\pm$	270	1,460	$\pm$	283	2,460	$\pm$	153
β -pinene	3,370	$\pm$	165	2,840	$\pm$	66	5,110	$\pm$	158
α -terpinene	2,720	$\pm$	79	689	$\pm$	20	469	$\pm$	17
terpinolene	1,550	$\pm$	22	649	$\pm$	17	669	$\pm$	29
menthone	1,040	$\pm$	117	7,170	$\pm$	794	449	$\pm$	49
<i>cis</i> - β -ocimene	906	$\pm$	16	361	$\pm$	12	1,200	$\pm$	50
<i>p</i> -cymene	625	$\pm$	17	958	$\pm$	86	175	$\pm$	6
α -thujene	434	$\pm$	33	198	$\pm$	6	486	$\pm$	11
camphene	130	$\pm$	8	117	$\pm$	3.2	175	$\pm$	4.2
β -thujene	52.8	$\pm$	4.8	87.7	$\pm$	2.1	22.3	$\pm$	0.7

^aCalculated as an average of peak areas $\pm$ standard deviation (n=3). * Presumed enantiomer based on literature.

Table 4.4 Concentrations of compounds quantified by GC-O dilution analysis

No. ^a	Compound	Concentration (µg/g; ppm)		
		Native	Scotch	Macho
10	1-hexen-3-one	0.10	1.63	0.81
11	3-methyl-2-butene-1-thiol	0.28	0.070	0.28
18	octanal	103	<103	824
19	1-octen-3-one	0.84	0.42	0.42
25	(3 <i>E</i> , 5 <i>Z</i>)-1,3,5-undecatriene	19.6	78.2	156
34	(<i>E,Z</i>)-2,6-nonadienal	332	166	332
40	phenylacetaldehyde	342	342	342
42	isovaleric acid	105	52.4	0.82
52	β-damascenone	5.82	2.91	2.91
56	2-phenylethanol	55.9	14.0	3.49
58	β-ionone	28.3	133	28.3
63	<i>p</i> -cresol	0.89	0.45	0.89
67	eugenol	1390	1390	2780
80	vanillin	0.34	0.34	0.34

^aNumbers refer to those in Tables 4.1-4.5 and Chapter 3.

Table 4.5 Concentrations, odor detection thresholds, and odor activity values for selected odorants in spearmint oils

Compound	Concentration (µg/g; ppm)			Threshold (ppb) ^a	OAV		
	Native	Scotch	Macho		Native	Scotch	Macho
R-(-)-carvone ^{c*}	752000	767000	670000	6.7 ^j	112,000,000	114,000,000	100,000,000
1,8-cineole ^c	19100	12400	12500	1.3 ^m	14,700,000	9,540,000	9,620,000
(E,Z)-2,6-nonadienal ^d	332	166	332	0.03 ^s	11,100,000	3,870,000	11,100,000
limonene ^c	97100	131000	117000	10 ^g	9,710,000	1,310,000	131,000
β-damascenone ^b	14.7	26.3	7.25	0.002 ^h	7,350,000	13,200,000	3,630,000
(3E,5Z)-1,3,5-undecatriene ^b	122	55.8	245	0.02 ^r	6,100,000	2,790,000	12,300,000
3-methylbutanal ^b	921	610	154	0.2 ^h	4,610,000	3,050,000	770,000
myrcene ^c	21300	8960	23000	13 ^e	1,640,000	689,000	1,770,000
ethyl-2-methyl butyrate ^b	216	125	177	0.15 ^o	1,440,000	833,000	1,180,000
β-ionone ^b	10.0	17.4	37.1	0.007 ^h	1,430,000	2,490,000	5,300,000
methyl-2-methyl butyrate ^b	356	201	172	0.25 ^o	1,420,000	804,000	688,000
2-methylpropanal ^b	534	315	80.7	1 ⁿ	534,000	315,000	80,700
α-pinene ^c	4180	5250	6880	10 ^t	418,000	525,000	688,000
octanal ^b	226	115	288	0.7 ^t	323,000	411,000	411,000
dimethyl sulfide ^b	71.2	27.3	10.9	0.3 ^h	237,000	91,000	36,300
3-methyl-2-butene-1-thiol ^d	0.28	0.070	0.28	0.0012 ^q	232,000	58,300	232,000
2-methylbutanal ^b	558	363	102	3 ⁿ	186,000	121,000	34,000
1-octen-3-one ^d	0.84	0.42	0.42	0.005 ^t	168,000	84,000	84,000
linalool ^b	733	649	507	6 ^t	122,000	108,000	84,500
eugenol ^b	475	285	320	6 ^t	70,900	42,500	47,800
ethyl-3-methyl butyrate ^b	14.7	6.57	13.6	0.2 ^o	73,500	32,900	68,000
nonanal ^b	71.2	181	206	1 ^t	71,200	181,000	206,000
p-cymene ^c	625	958	175	11.4 ^t	54,800	84,000	15,400
phenylacetaldehyde ^b	219	147	244	4 ^g	54,800	36,800	61,000
4-terpineol ^c	12700	3200	1690	340 ^e	37,400	9,410	4,970
β-pinene ^c	3370	2840	5110	140 ^k	24,100	20300	36,500
terpinolene ^c	1550	649	669	200 ^e	7,750	3250	3,350
menthone ^c	1040	7170	449	170 ^u	6,120	42,200	2,640
hexanal ^b	26.3	29.1	23.9	4.5 ^f	5,840	6,470	5,310
1-hexen-3-one ^d	0.10	1.63	0.81	0.02 ^t	5,000	81,500	40,500
3-methyl-1-butanol ^b	221	116	-	250 ^f	884	464	-
2-methyl-1-butanol ^b	188	88.1	-	300 ⁱ	627	294	-
2-phenylethanol ^b	224	307	152	1000 ^l	224	307	152
isovaleric acid ^d	105	52.4	0.82	540 ^o	194	97.0	1.52
2-methyl-1-propanol ^b	104	44.8	12.7	1000 ^p	104	44.8	12.7
p-cresol ^d	0.89	0.45	0.89	55 ^g	16.2	8.18	16.2
vanillin ^d	0.34	0.34	0.34	58 ⁱ	5.86	6.00	6.00

^aAroma threshold in water. ^bQuantified by SIDA. ^cQuantified by GC-FID. ^dQuantified by GC-O dilution analysis.

^eButtery et. al., 1968. ^fButtery et. al., 1978. ^gButtery et. al., 1988. ^hButtery et. al., 1990. ⁱButtery and Ling, 1995. ^jDu et. al., 2010. ^kPlotto et. al., 2004. ^lSchieberle, 1991. ^mButtery et. al., 1987. ⁿMilo and Grosch, 1993. ^oButtery et. al., 1997. ^pSchieberle and Hofmann, 1997. ^qJørgensen et. al., 2001. ^rFritsch and Schieberle, 2005. ^sTokitomo et. al., 2005. ^tGreger and Schieberle, 2007. ^uAhmed et. al., 1978. ^vAmoore and Venstrom, 1966. ^wPresumed enantiomer based on literature.

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CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH

Spearmint has been grown in gardens since the 9th century as an herb thought to possess a wide range of health benefits. Today, spearmint is grown over 24,500 acres of land and owes its popularity to its use in chewing gum and breath mints, as well as other confectionery products and oral healthcare products. The main volatile constituents in Native and Scotch spearmint oils are well known. The most abundant compound in both is R-(-)-carvone, which has a distinctive spearmint-like aroma. While the major volatiles in spearmint are well-characterized, little research has been performed on the analysis of odorants in spearmint oils. Only two studies in the past literature have directly analyzed the odorants in Native spearmint oil. Therefore, the first objective of this research was to identify the potent odorants in spearmint oil using gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). The second objective was to develop methods to accurately and precisely quantify the trace-level potent odorants to determine their odor activity values. Furthermore, it was hypothesized that there are trace level odorants, both known and unknown, that contribute to the aroma of spearmint.

Native spearmint, Scotch spearmint, and Macho mint oils were analyzed using GC-O and GC-MS to identify potent odor-active compounds. Eighty-five odorants were detected among the three spearmint oils. Of the 85 odorants, 41 were positively identified, four were tentatively identified, and the identities of 40 odorants remain unknown. The relative potencies of the odor-active compounds were determined using aroma extract dilution analysis (AEDA). The most potent odorant in all three spearmint oils was R-(-)-carvone. Other highly potent odorants were

eugenol (clove), ethyl 2-methylbutyrate (fruity), β -damacenone (applesauce), and (3*E*,5*Z*)-1,3,5-undecatriene (tape). Several potent odorants were identified that had not been previously reported in spearmint, including 1-hexen-3-one (plastic), 3-methyl-2-butene-1-thiol (skunky), and 2-methylisoborneol (earthy).

A variety of methods were used to determine the concentration of 46 odorants in the spearmint oils. Stable isotope dilution analysis (SIDA) was the most accurate and precise quantification method and was used for the quantification of 20 potent odorants. Gas chromatography-flame ionization detection (GC-FID) was used to quantify 19 compounds present in high abundance. GC-O dilution analysis was used to quantify 14 compounds. While this technique has lower accuracy and precision than other quantification methods, it is the only viable method to quantify potent odorants present in trace levels.

The concentrations of the potent odorants were used to determine the odor activity value (OAV) of each compound. The OAV value indicates the potency of each compound in a sample matrix, unlike FD factors which indicate the relative potency of the individual compounds in air. For all three spearmint oils, the compound with the highest OAV was R-(-)-carvone, followed by 1,8-cineole (eucalyptus), (*E,Z*)-2,6-nonadienal (cucumber), β -damascenone, and (3*E*,5*Z*)-1,3,5-undecatriene. These results agree with previous literature in that R-(-)-carvone is the most important odorant in spearmint oil. Furthermore, many of the compounds with high OAVs had not been previously reported in spearmint oil, such as (*E,Z*)-2,6-nonadienal and β -damascenone.

Over the course of these studies, several new methods were developed to quantify potent odorants in the spearmint oils. SIDA was used in combination with solvent extraction techniques to remove compounds coeluting with the analyte. For analytes coeluting with each other, GC

conditions were modified to resolve the compounds. Compounds in high abundance were quantified using GC-FID, where the peak areas of several analytes were compared to that of an internal standard. In contrast, compounds very low in abundance, but still visible on a GC-MS, were quantified using selected ion monitoring (SIM) rather than total ion current (TIC) mode on the mass spectrophotometer to increase the sensitivity of the ions selected for quantitation. Trace potent odorants which are not visible on a GC-MS were quantified using GC-O dilution analysis. The rationale for quantitation method selection can be seen in Figure 5.1.

While these studies provide a firm understanding of the potent odorants in spearmint oils, further research could be performed to gain additional information on the flavor chemistry of spearmint oils. One important additional study would be the determination of enantiomeric ratios of certain potent odorants in spearmint oils. Several enantiomeric compounds exist in spearmint oils. Among these are the potent odorants carvone, limonene, and ethyl 2-methylbutyrate. In this thesis, carvone was assumed to exist in only the R enantiomeric form. As carvone is the major constituent of the three spearmint oils, it would be beneficial to determine the exact enantiomeric ratio of carvone in each of the spearmint oils. It would also be advantageous to find the enantiomeric ratio of limonene and ethyl-2-methyl butyrate, since they also have high OAVs.

Furthermore, several trace potent odorants were identified, but could not be quantified by SIDA since they were undetectable using GC-MS. Some of these trace potent odorants were quantified using GC-O dilution analysis, which is not as accurate or precise a quantification method as SIDA. The development of enrichment techniques would be beneficial for the detection of these compounds by GC-MS. Additionally, the synthesis of the corresponding labeled isotopes also would be conducted for quantification of the trace potent odorants by SIDA.

Finally, the understanding of spearmint flavor could be enhanced by the identification of unknown odorants in the spearmint oils. Many of the potent odorants in the spearmint oils exist in trace levels and therefore are difficult to identify. Enrichment or extraction methods and advanced chromatography techniques would be necessary to isolate and identify the unknown odorants.

5.1 FIGURES AND TABLES

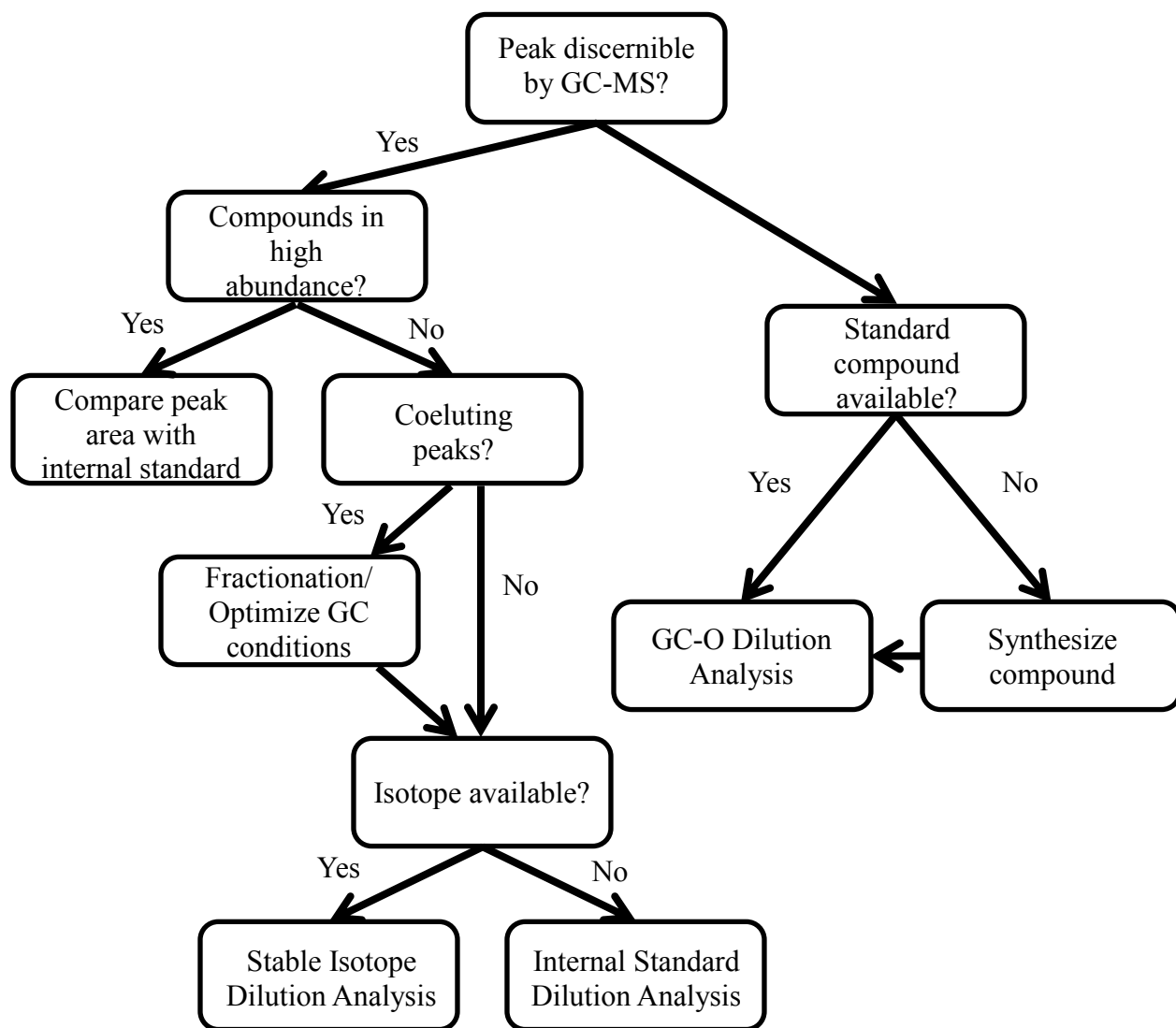


Figure 5.1 Flow diagram of quantitation method selection

APPENDIX A

QUANTITY OF LABELED ISOTOPES FOR STABLE ISOTOPE DILUTION ANALYSIS

Analyte ^a		Labeled Isotope ^b		Concentration (mg/mL)	Volume (μL)
eugenol	(67)	[² H ₃]-eugenol	(I-67)	1.0	10
linalool	(33)	[² H ₂]-linalool	(I-33)	1.1	20
hexanal	(9)	[² H ₄]-octanal	(I-18)	1.1	4
octanal	(18)				
nonanal	(24)	[² H ₄]-nonanal	(I-24)	1.2	4
dimethyl sulfide	(1)	[² H ₆]-dimethyl sulfide	(I-1)	11.6	3
2-methylpropanal	(2)	[² H ₂]-3-methylbutanal	(I-4)	40.0	3
3-methylbutanal	(4)				
2-methylbutanal	(3)				
2-methyl-1-propanol	(81)	[² H ₂]-2-methyl-1-propanol	(I-81)	10.2	3
3-methyl-1-butanol	(82)	[² H ₂]-3-methyl-1-butanol	(I-82)	9.9	3
2-methyl-1-butanol	(83)	[² H ₂]-2-methyl-1-butanol	(I-83)	10.9	3
β-damascenone	(52)	[² H ₄]-β-damascenone	(I-52)	0.98	4
β-ionone	(58)	[² H ₃]-β-ionone	(I-58)	1.17	2
(3E,5Z)-1,3,5-undecatriene	(25)	[² H ₄]- (3E,5Z)-1,3,5-undecatriene	(I-25)	4.0	4
phenylacetaldehyde	(40)	[¹³ C ₂]-phenylacetaldehyde	(I-40)	6.56	2
2-phenylethanol	(56)	[¹³ C ₂]-2-phenylethanol	(I-56)	0.98	2
methyl-2-methyl butyrate	(5)	[² H ₃]-ethyl-3-methyl butyrate	(I-8)	1.97	3
ethyl-2-methyl butyrate	(7)				
ethyl-3-methyl butyrate	(8)				

^aNumbers refer to those in Tables 4.1-4.5 and Chapter 3. ^bNumbers refer to those in Figure 4.1.

APPENDIX B

QUANTIFICATION DATA

Eugenol			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	492.97	282.30	332.98
2	497.21	293.29	319.27
3	434.18	278.59	306.69
Average	474.79	284.73	319.65
Standard Deviation	35.23	7.65	13.15

Linalool			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	769.14	635.86	508.27
2	743.81	638.77	548.51
3	686.36	674.34	465.13
Average	733.10	649.66	507.31
Standard Deviation	42.42	21.42	41.70

Hexanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	26.57	26.31	24.44
2	30.85	37.98	28.11
3	21.40	22.90	19.12
Average	26.27	29.06	23.89
Standard Deviation	4.73	7.91	4.52

Octanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	240.80	117.92	287.43
2	231.65	123.82	306.73
3	206.04	103.95	268.92
Average	226.16	115.23	287.69
Standard Deviation	18.02	10.20	18.90

Nonanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	80.43	166.54	199.88
2	75.22	203.50	219.17
3	57.93	171.70	200.40
Average	71.19	180.58	206.49
Standard Deviation	11.78	20.02	10.99

Dimethyl sulfide			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	71.37	30.65	11.21
2	72.18	23.84	10.01
3	70.09	27.47	11.62
Average	71.21	27.32	10.95
Standard Deviation	1.05	3.41	0.839
2-Methyl propanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	504.68	291.02	70.87
2	502.63	318.47	81.43
3	595.61	335.24	89.74
Average	534.31	314.91	80.68
Standard Deviation	53.10	22.33	9.45
3-Methyl butanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	891.60	578.46	146.26
2	826.52	595.33	165.78
3	1045.97	656.70	148.63
Average	921.36	610.17	153.56
Standard Deviation	112.71	41.18	10.65
2-Methyl butanal			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	528.72	339.12	97.12
2	554.85	356.69	103.14
3	590.02	393.32	104.97
Average	557.86	363.05	101.74
Standard Deviation	30.76	27.65	4.11
2-Methyl-1-propanol			
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	104.73	47.25	13.37
2	103.17	45.84	10.73
3	103.92	41.31	13.95
Average	103.94	44.80	12.68
Standard Deviation	0.780	3.10	1.71

3-Methyl-1-butanol

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	229.25	117.60	-
2	217.63	111.81	-
3	214.89	117.46	-
Average	220.59	115.62	-
Standard Deviation	7.63	3.31	-

2-Methyl-1-butanol

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	203.07	88.01	-
2	183.10	85.98	-
3	177.77	90.39	-
Average	187.98	88.13	-
Standard Deviation	13.34	2.21	-

β -Damascenone

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	16.62	29.15	7.85
2	11.65	25.60	7.21
3	15.90	24.26	6.68
Average	14.72	26.34	7.25
Standard Deviation	2.69	2.52	0.588

β -Ionone

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	9.22	18.32	39.31
2	5.00	17.13	33.96
3	15.79	16.60	38.00
Average	10.01	17.35	37.09
Standard Deviation	5.43	0.882	2.79

(3E,5Z)-1,3,5-Undecatriene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	124.6	60.2	249.4
2	115.8	58.5	243.2
3	126.2	48.7	241.8
Average	122.2	55.8	244.8
Standard Deviation	5.62	6.21	4.05

Phenylacetaldehyde

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	187.6	152.3	216.6
2	235.7	154.8	269.7
3	234.0	132.3	245.0
Average	219.1	146.5	243.8
Standard Deviation	27.3	12.3	26.6

2-Phenylethanol

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	215.0	309.3	154.4
2	220.4	304.4	147.5
3	236.0	307.8	152.7
Average	223.8	307.1	151.5
Standard Deviation	10.9	2.54	3.59

Methyl-2-methyl butyrate

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	417.8	221.8	196.4
2	339.4	203.0	173.0
3	311.3	176.6	146.2
Average	356.2	200.5	171.8
Standard Deviation	55.2	22.7	25.1

Ethyl-2-methyl butyrate

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	240.1	135.1	184.9
2	224.4	127.2	189.0
3	183.1	112.8	158.1
Average	215.9	125.1	177.3
Standard Deviation	29.5	11.3	16.8

Ethyl-3-methyl butyrate

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	15.3	7.31	14.0
2	16.2	6.49	14.5
3	12.8	5.91	12.3
Average	14.7	6.57	13.6
Standard Deviation	1.84	0.70	1.16

α -Thujene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	458	191	497
2	396	201	475
3	448	201	486
Average	434	198	486
Standard Deviation	32.8	6.10	10.9

 α -Pinene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	4336	5093	7027
2	3848	5297	6720
3	4364	5342	6886
Average	4183	5244	6878
Standard Deviation	290.1	133.0	153.4

Camphene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	135	114	180
2	121	119	171
3	135	119	175
Average	130	117	175
Standard Deviation	7.8	3.2	4.2

 β -Thujene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	57	85	23
2	48	89	21
3	53	89	23
Average	53	88	22
Standard Deviation	4.8	2.1	0.7

 β -Pinene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	3442	2762	5291
2	3178	2880	4987
3	3481	2873	5065
Average	3367	2838	5115
Standard Deviation	164.6	66.0	157.7

Myrcene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	21517	8735	23955
2	20539	9100	22474
3	21833	9042	22607
Average	21296	8959	23012
Standard Deviation	675.0	196.3	819.0

α -Terpinene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	2742	666	488
2	2633	703	458
3	2785	698	460
Average	2720	689	469
Standard Deviation	78.5	20.3	16.7

p-Cymene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	633	859	182
2	605	1008	175
3	637	1007	170
Average	625	958	175
Standard Deviation	17.0	85.6	5.6

Limonene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	97562	127411	122296
2	94545	133540	114356
3	99229	132709	114456
Average	97112	131220	117036
Standard Deviation	2374.2	3325.0	4555.7

cis- β -Ocimene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	906	348	1257
2	890	370	1176
3	922	365	1166
Average	906	361	1200
Standard Deviation	16.1	11.6	50.1

γ -Terpinene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	4530	1169	873
2	4457	1231	815
3	4608	1220	811
Average	4532	1207	833
Standard Deviation	76.0	32.7	34.7

Terpinolene

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	1544	630	702
2	1529	660	655
3	1572	658	651
Average	1548	649	669
Standard Deviation	21.9	16.8	28.6

R-(-)-Carvone

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	771531	767087	661937
2	743642	776936	685892
3	739998	755583	663337
Average	751724	766535	670389
Standard Deviation	17249.8	10687.4	13444.7

1,8-Cineole

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	18790	12061	12682
2	19116	12827	12472
3	19503	12255	12222
Average	19136	12381	12459
Standard Deviation	356.8	398.0	230.0

3-Octyl acetate

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	3135	1304	2412
2	3665	1791	2628
3	3486	1298	2332
Average	3429	1464	2457
Standard Deviation	269.6	282.8	153.3

Menthone

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	929	6712	431
2	1163	8092	504
3	1037	6721	412
Average	1043	7175	449
Standard Deviation	117.4	794.0	48.8

4-Terpineol

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	11338	3286	1620
2	14202	3473	1846
3	12615	2833	1612
Average	12718	3197	1693
Standard Deviation	1434.5	328.8	133.0

Dihydrocarvyl acetate

Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	3384	872	7193
2	4794	1147	8809
3	3934	897	6623
Average	4037	972	7542
Standard Deviation	710.5	152.1	1133.7

E-Carvyl acetate

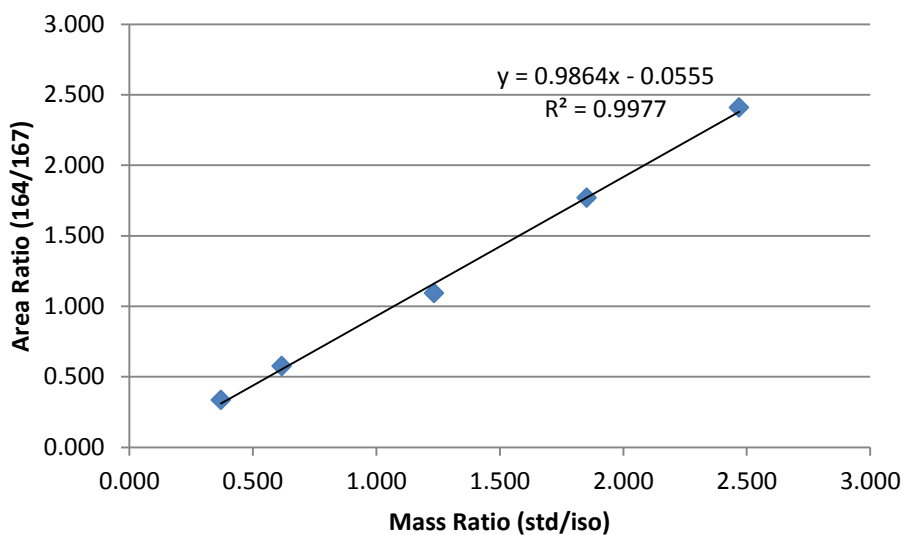
Replication	Concentration (ppm)		
	Native	Scotch	Macho
1	3868	1292	6534
2	5779	1792	8200
3	4454	1286	6012
Average	4700	1457	6915
Standard Deviation	979.1	290.5	1142.8

APPENDIX C

CALIBRATION CURVES

Response factor of eugenol against [2H_3]-eugenol

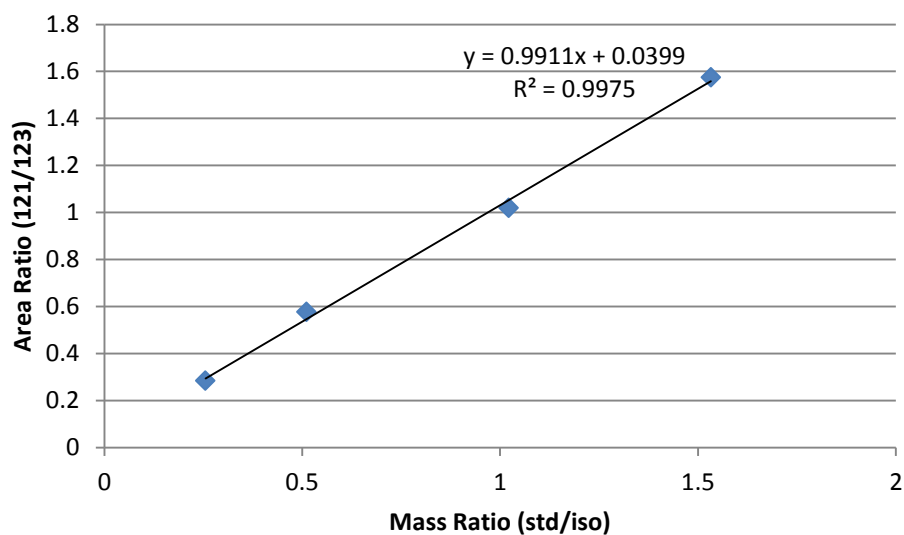
		Unlabeled	Isotope	Area Ratio
Selected ion:		164	167	
Mass ratio:	0.370	3918623	11775740	0.333
	0.617	6761678	11764556	0.575
	1.234	12491765	11439538	1.092
	1.852	19389881	10961456	1.769
	2.469	24534866	10190454	2.408



Slope: 0.9864
Response factor: 1.014

Response factor of linalool against [2H_2]-linalool

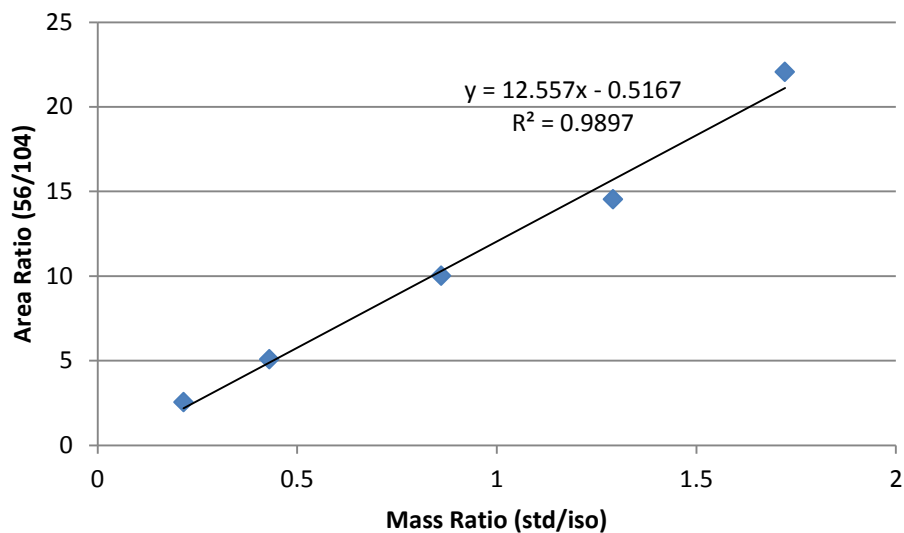
		Unlabeled	Isotope	Area Ratio
Selected ion:		121	123	
Mass ratio:	0.2554318	683496	2415381	0.282976475
	0.5108636	1375149	2387282	0.576031236
	1.0217272	1599725	1571438	1.01800071
	1.5325908	3333908	2118730	1.573540753



Slope: 0.9911
Response factor: 1.009

Response factor of hexanal against [2H_2]-octanal

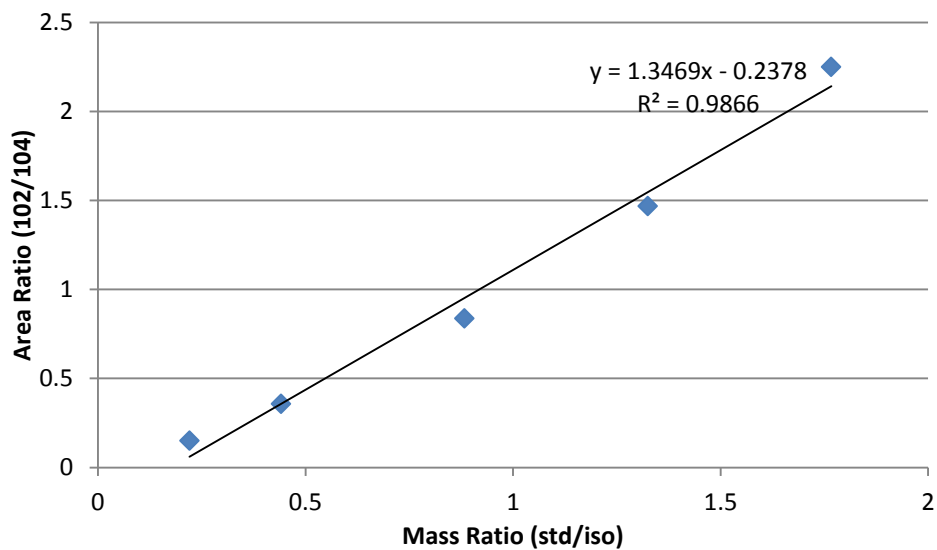
		Unlabeled	Isotope	Area Ratio
Selected ion:		56	104	
Mass ratio:	0.21525725	6445493	2547817	2.52981003
	0.4305145	12446276	2453984	5.071865179
	0.861029	25777290	2575371	10.00915596
	1.2915435	44807615	3085674	14.52117592
	1.722058	52424515	2378035	22.04530842



Slope: 12.557
Response factor: 0.0796

Response factor of octanal against [2H_2]-octanal

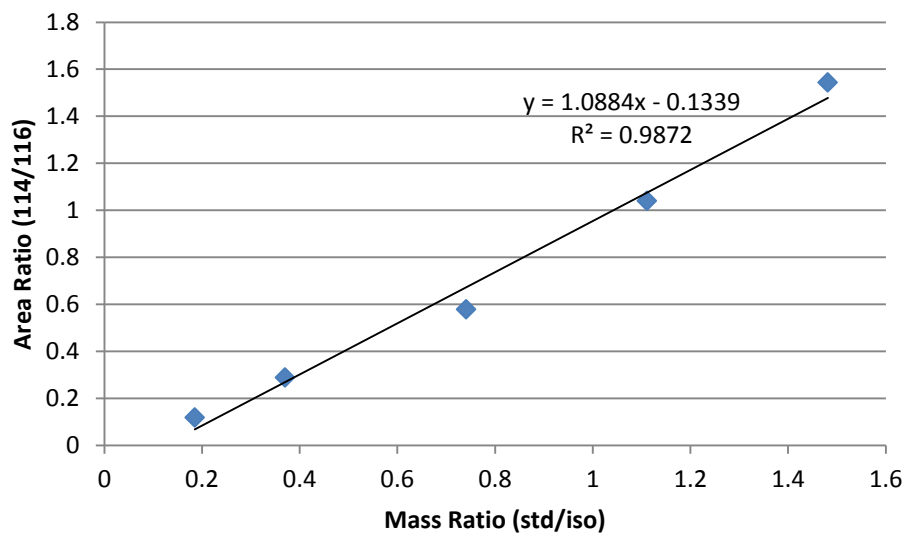
		Unlabeled	Isotope	Area Ratio
Selected ion:		102	104	
Mass ratio:	0.220852273	381905	2547817	0.149894989
	0.441704545	873922	2453984	0.356123756
	0.883409091	2152090	2575371	0.835642709
	1.325113636	4527629	3085674	1.467306332
	1.766818182	5348104	2378035	2.24895933



Slope: 1.3469
Response factor: 0.742

Response factor of nonanal against [2H_2]-nonanal

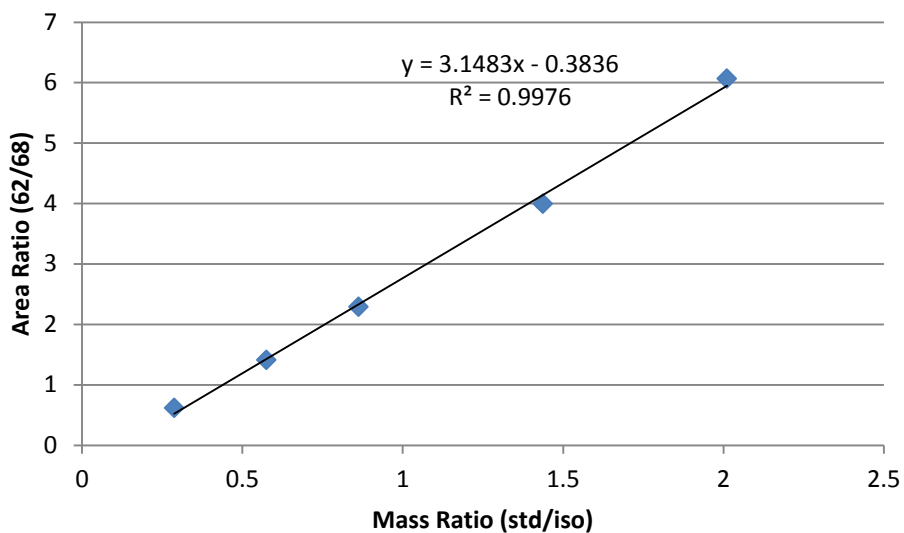
		Unlabeled	Isotope	Area Ratio
Selected ion:		114	116	
Mass ratio:	0.185186	271889	2325081	0.116937431
	0.370372	630872	2195277	0.287376946
	0.740744	1533787	2657156	0.577228812
	1.111116	3195228	3076887	1.038461276
	1.481488	3638126	2357718	1.543070885



Slope: 1.0884
Response factor: 0.919

Response factor of dimethyl sulfide against [2H_6]-dimethyl sulfide

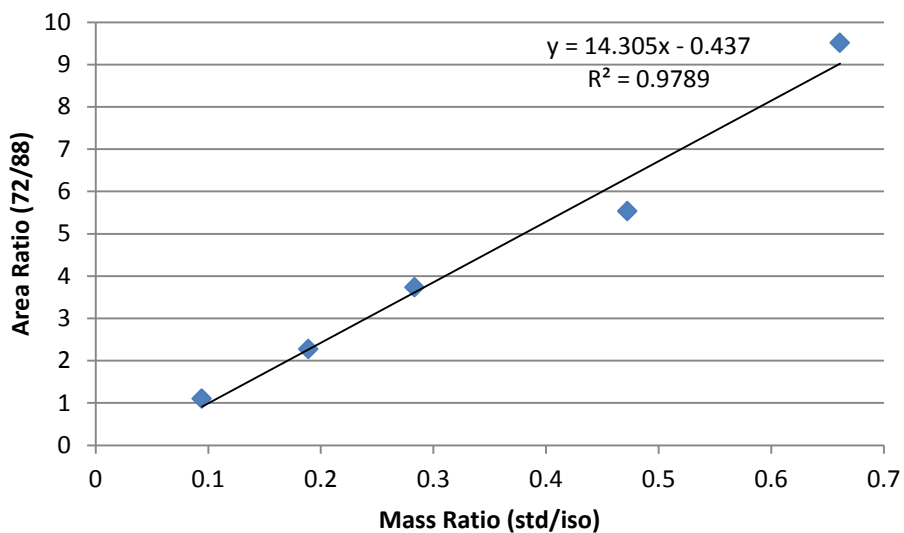
		Unlabeled	Isotope	Area Ratio
Selected ion:		62	68	
Mass ratio:	0.287344828	836646	1363290	0.613696279
	0.574689655	1584490	1125312	1.408045058
	0.862034483	2010846	879451	2.286478724
	1.436724138	3447594	863194	3.993996715
	2.011413793	3484866	574708	6.063715835



Slope: 3.1483
Response factor: 0.318

Response factor of 2-methyl propanal against [2H_2]-3-methyl butanal

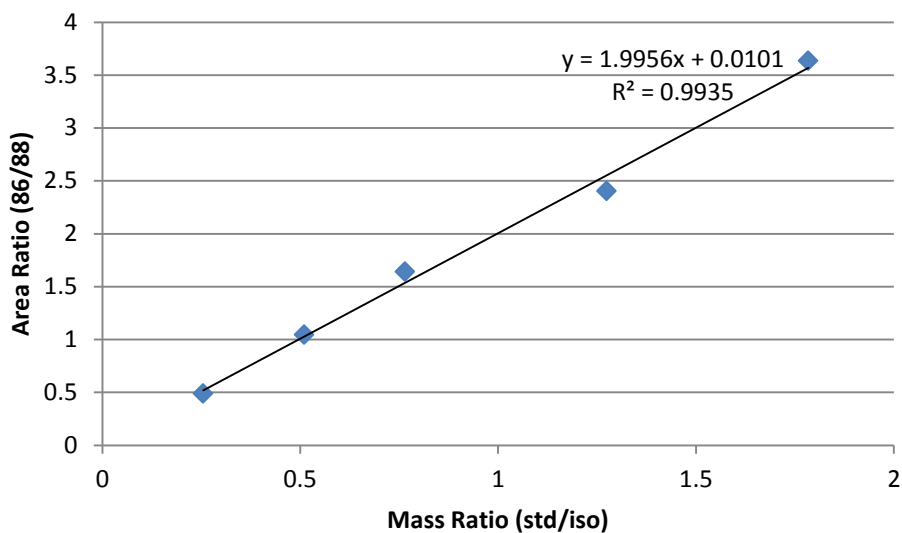
		Unlabeled	Isotope	Area Ratio
Selected ion:		72	88	
Mass ratio:	0.094452033	224157	204498	1.09613297
	0.188904067	420090	184867	2.272390421
	0.2833561	618376	165759	3.730572699
	0.472260167	949108	171727	5.526842023
	0.661164233	956636	100595	9.509776828



Slope: 14.305
Response factor: 0.0699

Response factor of 3-methyl butanal against [2H_2]-3-methyl butanal

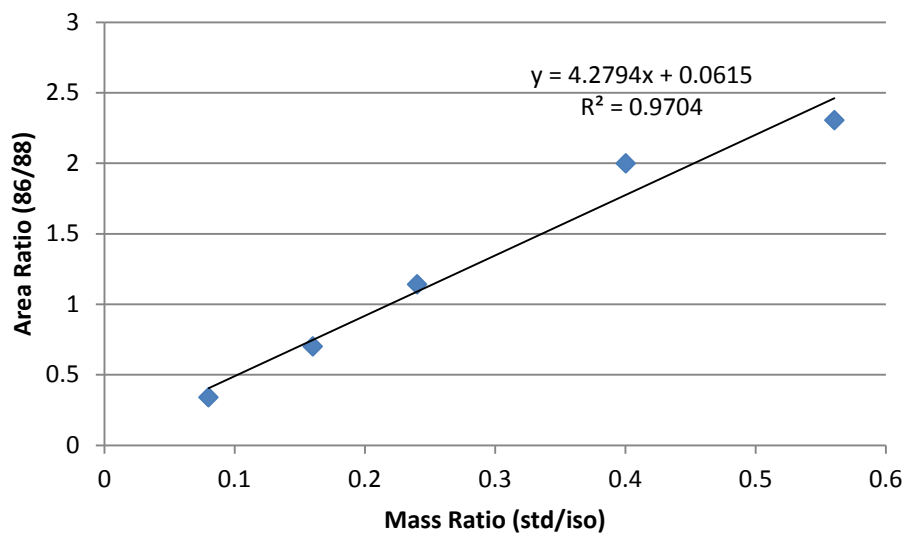
		Unlabeled	Isotope	Area Ratio
Selected ion:		86	88	
Mass ratio:	0.254842783	99342	204498	0.485784702
	0.509685567	192993	184867	1.043955925
	0.76452835	271852	165759	1.640043678
	1.274213917	412394	171727	2.40145114
	1.783899483	365509	100595	3.633470848



Slope: 1.9956
Response factor: 0.501

Response factor of 2-methyl butanal against [2H_2]-3-methyl butanal

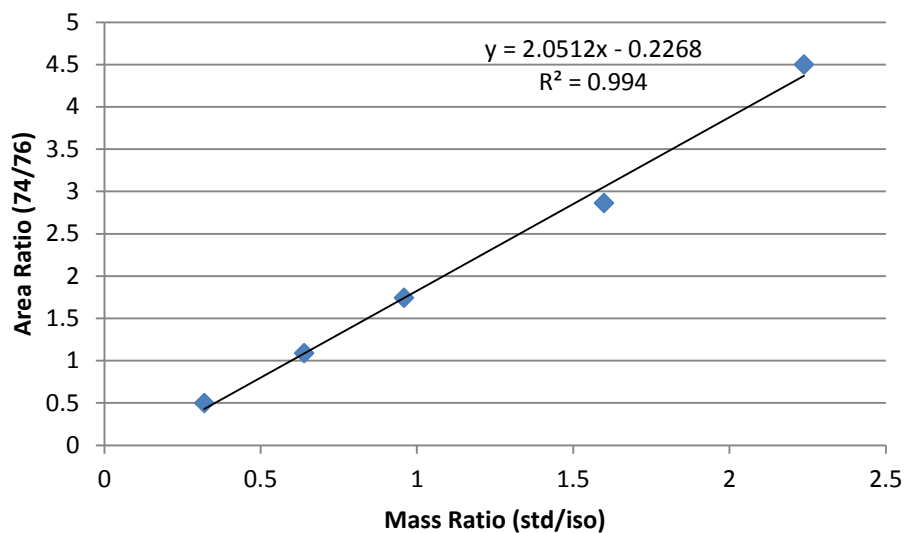
		Unlabeled	Isotope	Area Ratio
Selected ion:		86	88	
Mass ratio:	0.080087417	69038	204498	0.337597434
	0.160174833	129020	184867	0.697907144
	0.24026225	188945	165759	1.139877774
	0.400437083	342935	171727	1.996977761
	0.560611917	231775	100595	2.304040956



Slope: 4.2794
Response factor: 0.234

Response factor of 2-methyl-1-propanol against [2H_2]-2-methyl-1-propanol

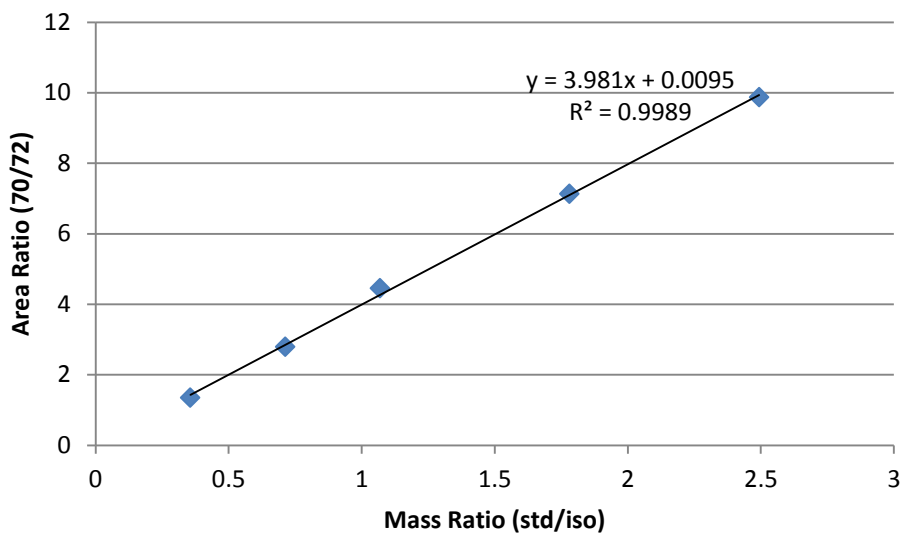
		Unlabeled	Isotope	Area Ratio
Selected ion:		74	76	
Mass ratio:	0.319832288	46154	92961	0.496487774
	0.639664575	92128	84903	1.085097111
	0.959496863	126648	72853	1.738404733
	1.599161438	230635	80691	2.858249371
	2.238826013	263650	58631	4.496767921



Slope: 2.0512
Response factor: 0.488

Response factor of 3-methyl-1-butanol against [2H_2]-3-methyl-1-butanol

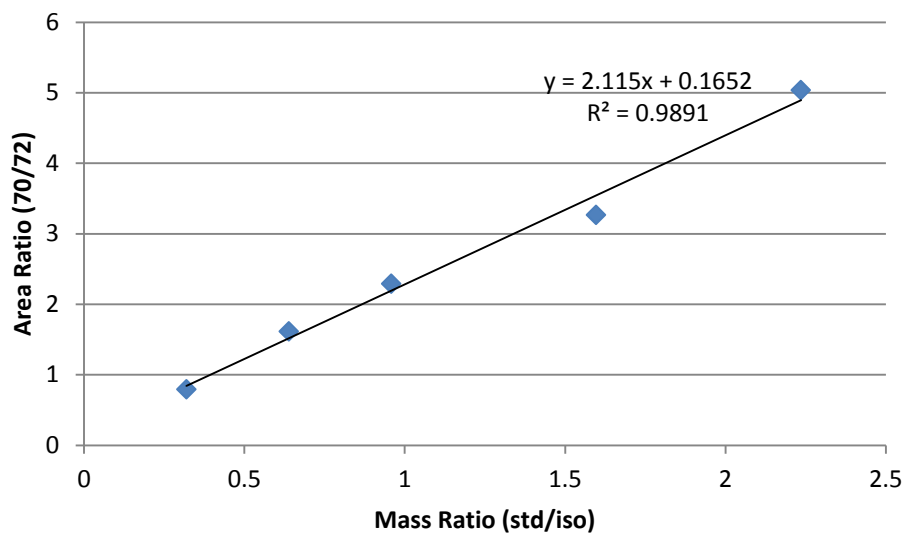
		Unlabeled	Isotope	Area Ratio
Selected ion:		70	72	
Mass ratio:	0.356288485	229852	170677	1.346707524
	0.71257697	472587	169628	2.786019997
	1.068865455	693534	155846	4.45012384
	1.781442424	1248486	175242	7.124353751
	2.494019394	1528794	154876	9.871083964



Slope: 3.981
Response factor: 0.251

Response factor of 2-methyl-1-butanol against [2H_2]-2-methyl-1-butanol

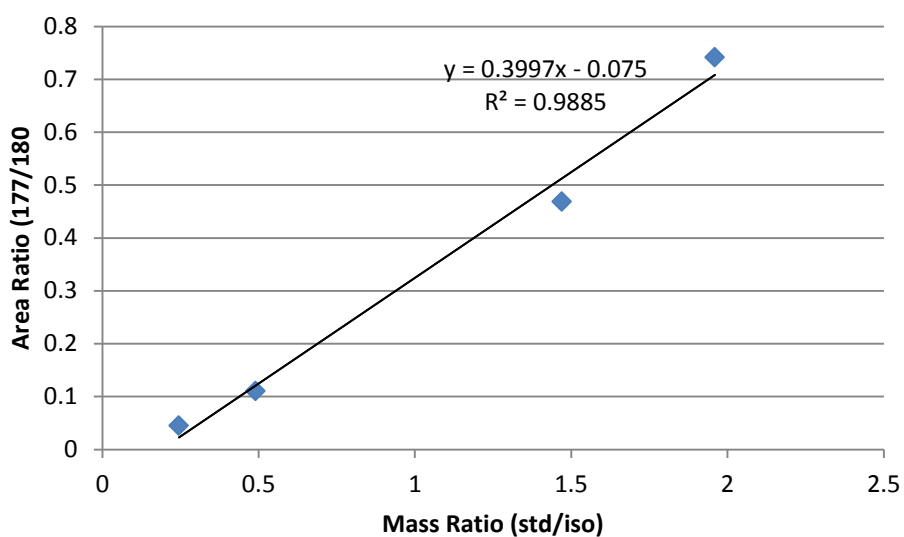
		Unlabeled	Isotope	Area Ratio
Selected ion:		70	72	
Mass ratio:	0.319399083	224145	283063	0.791855523
	0.638798165	448881	278898	1.609480885
	0.958197248	564957	247014	2.287145668
	1.596995413	882594	270302	3.265214464
	2.235793578	1094583	217546	5.031501384



Slope: 2.115
Response factor: 0.473

Response factor of β -damascenone against [2H_4]- β -damascenone

		Unlabeled	Isotope	Area Ratio
Selected ion:		190	194	
Mass ratio:	0.24495695	890003	19852120	0.044831635
	0.4899139	2289918	20819451	0.109989356
	1.469741701	13663418	29170591	0.468397024
	1.959655601	17481745	23578335	0.741432548

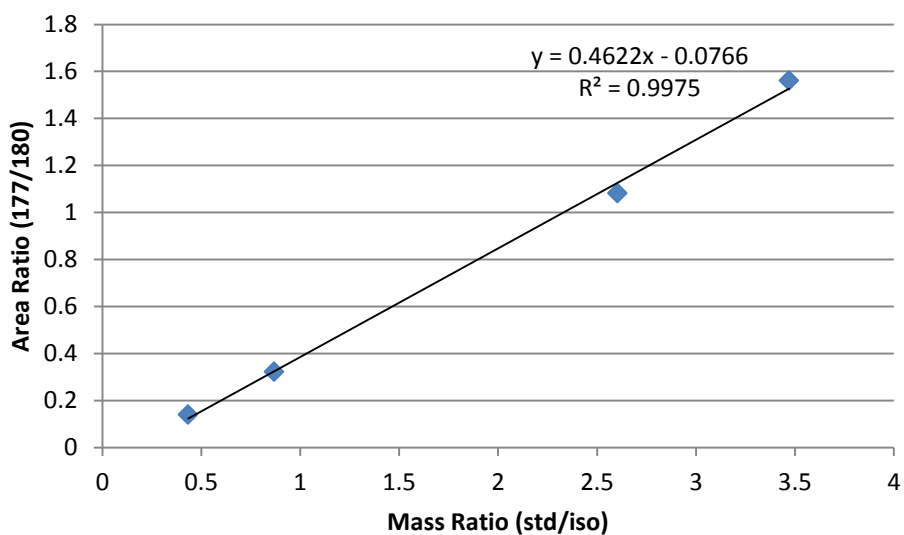


Slope: 0.3997

Response factor: 2.502

Response factor of β -ionone against [2H_3]- β -ionone

		Unlabeled	Isotope	Area Ratio
Selected ion:		177	180	
Mass ratio:	0.433826667	7004105	50185916	0.13956316
	0.867653333	17217827	53617065	0.321125877
	2.60296	82532794	76308147	1.081572509
	3.470613333	99249813	63625044	1.559917397

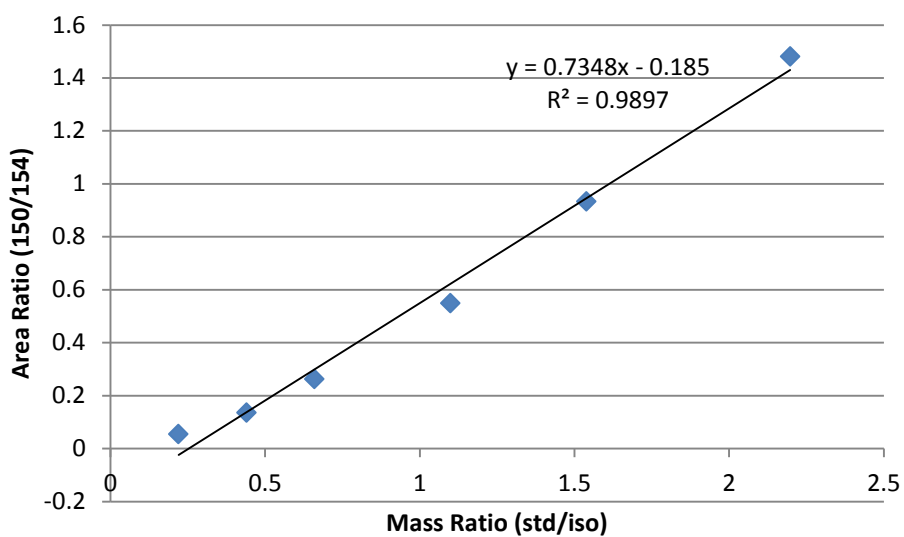


Slope: 0.4622

Response factor: 2.164

Response factor of (3E,5Z)-1,3,5-undecatriene against [²H₄]- (3E,5Z)-1,3,5-undecatriene

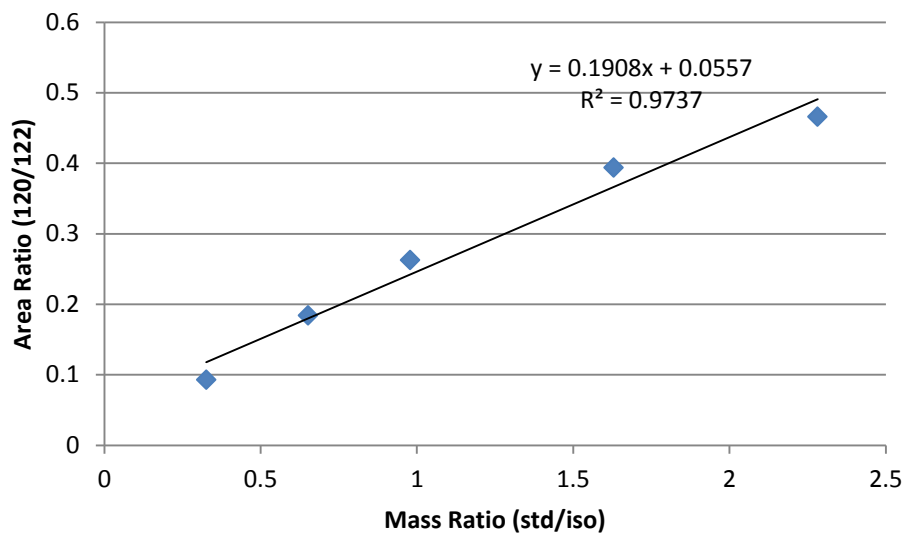
		Unlabeled	Isotope	Area Ratio
Selected ion:		150	154	
Mass ratio:	0.2197691	4783725	88285188	0.05418491
	0.4395382	11950885	88817685	0.134555241
	0.6593073	26992921	103196779	0.261567476
	1.0988455	39307691	71701263	0.548214764
	1.5383837	66222722	70969384	0.933116765
	2.197691	97536894	65913363	1.4797742



Slope: 0.7348
Response factor: 1.361

Response factor of phenylacetaldehyde against [$^{13}\text{C}_2$]- phenylacetaldehyde

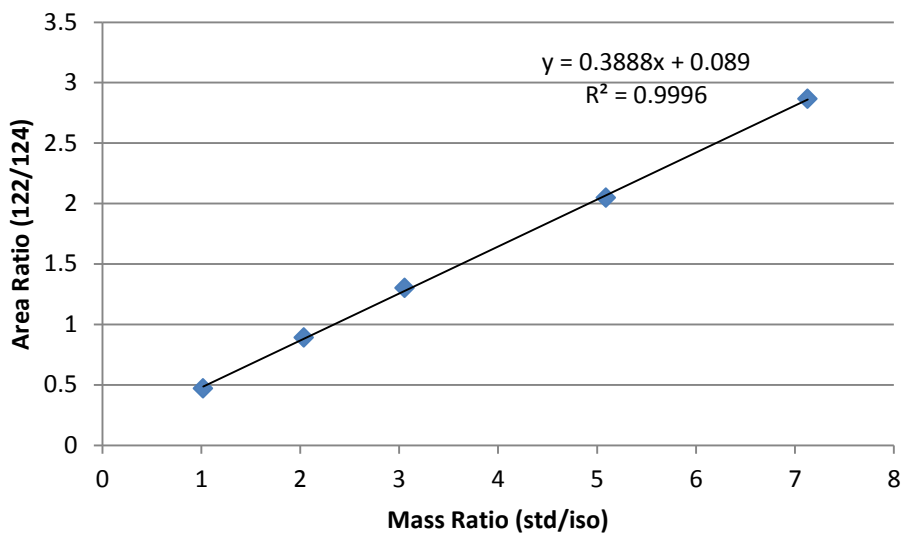
		Unlabeled	Isotope	Area Ratio
Selected ion:		120	122	
Mass ratio:	0.32601275	23716876	256175505	0.092580577
	0.652025499	47406645	257885328	0.183828391
	0.978038249	80433696	306807973	0.262162991
	1.630063749	97382320	247348826	0.393704395
	2.282089248	112207380	240892689	0.465798196



Slope: 0.1908
Response factor: 5.241

Response factor of 2-phenylethanol against [$^{13}\text{C}_2$]- 2-phenylethanol

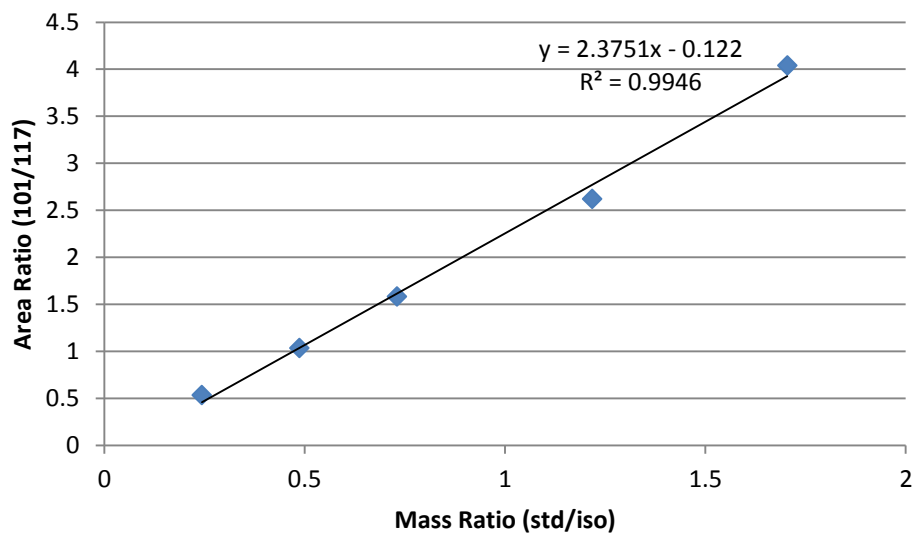
Selected ion:		Unlabeled	Isotope	Area Ratio
		122	124	
Mass ratio:	1.018316327	22291097	47533351	0.468956986
	2.036632653	47539869	53444449	0.889519303
	3.05494898	86164602	66228563	1.301018746
	5.091581633	108585054	53058221	2.046526475
	7.128214286	141274929	49297057	2.865788296



Slope: 0.3888
Response factor: 2.572

Response factor of methyl-2-methyl butyrate against [2H_2]-ethyl-3-methyl butyrate

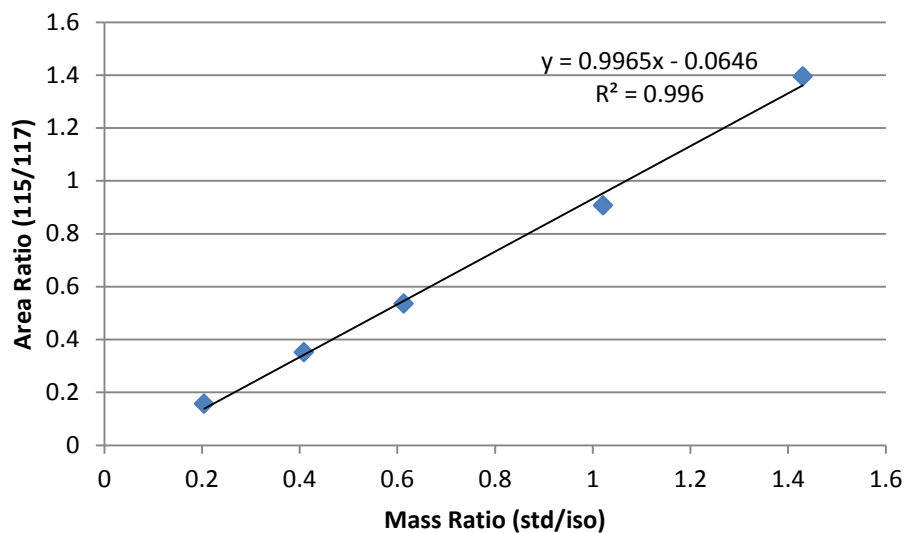
Selected ion:		Unlabeled	Isotope	Area Ratio
		101	117	
Mass ratio:	0.243479391	2080649	3912272	0.531826264
	0.486958782	3096443	3005248	1.030345249
	0.730438173	7759843	4910302	1.580318889
	1.217396954	12125450	4630494	2.618608295
	1.704355736	22640125	5606579	4.038135376



Slope: 2.3751
Response factor: 0.421

Response factor of ethyl-2-methyl butyrate against [2H_2]-ethyl-3-methyl butyrate

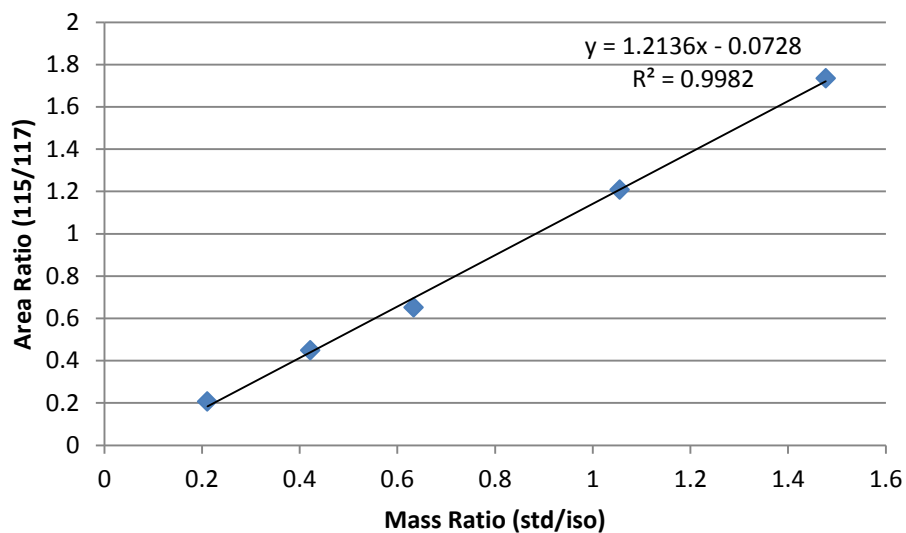
		Unlabeled	Isotope	Area Ratio
Selected ion:		115	117	
Mass ratio:	0.204301641	609155	3912272	0.155703642
	0.408603283	1053191	3005248	0.350450612
	0.612904924	2627322	4910302	0.53506322
	1.021508206	4194963	4630494	0.905942865
	1.430111489	7818620	5606579	1.394543803



Slope: 0.9965
Response factor: 1.004

Response factor of ethyl-3-methyl butyrate against [2H_2]-ethyl-3-methyl butyrate

		Unlabeled	Isotope	Area Ratio
Selected ion:		115	117	
Mass ratio:	0.211055415	805797	3912272	0.205966507
	0.422110829	1346759	3005248	0.448135728
	0.633166244	3197684	4910302	0.651219416
	1.055277073	5592230	4630494	1.207696198
	1.477387902	9720931	5606579	1.733843579



Slope: 1.2136
Response factor: 0.824

APPENDIX D

GC-O DILUTION ANALYSIS QUANTITATION DATA

Compound	Concentration in Solution (µg/g; ppm)	Dilution Threshold in Solution	Dilution Threshold in Oil			Concentration (µg/g; ppm)		
			Native	Scotch	Macho	Native	Scotch	Macho
1-hexen-3-one	0.102	8	8	128	64	0.10	1.63	0.81
3-methyl-2-butene- 1-thiol	570	1048576	2048	64	2048	0.278	0.070	0.278
octanal	103	8	8	128	64	103	<103	824
1-octen-3-one	0.105	32	256	128	128	0.84	0.42	0.42
(3 <i>E</i> ,5 <i>Z</i>)-1,3,5- undecatriene	39.1	8192	4096	1024	32768	19.6	78.2	156
(<i>E</i> , <i>Z</i>)-2,6- nonadienal	10.4	64	2048	1024	2048	332	166	332
phenylacetaldehyde	10.7	32	1024	512	1024	342	342	342
isovaleric acid	105	1024	1024	4096	8	105	52.4	0.82
β-damascenone	0.0114	16	8192	1024	4096	5.82	2.91	2.91
phenylethanol	112	4096	32	-	32	0.87	<0.22	0.87
β-ionone	113	1024	256	1024	256	28.3	133	28.3
<i>p</i> -cresol	114	16384	128	64	128	0.89	0.45	0.89
eugenol	10.9	512	65536	65536	131072	1390	1390	2780
vanillin	10.8	2048	64	64	64	0.34	0.34	0.34