GINGER *(Zingiber officinale)*:

ANTIOXIDANTS AND THEIR USE IN STABILIZING LEMON OIL

by

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ABSTRACT OF THE DISSERTATION

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Antioxidants and Their Use in Stabilizing Lemon Oil

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Nine ginger powders from various parts of the world have been studied for their antioxidant strength by way of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC). Of those tested, it was found that the acetone extract of Nigerian ginger from Synthite had the greatest antioxidant power. The extract was fractionated using the SepBox® and each fraction collected was tested for its ORAC value. The fractions with the greatest ORAC values are listed in increasing order, and were identified and confirmed by LC/MS/UV, MS/MS and HRMS: [6]-gingerdiol, octahydrocurcumin, [6]-gingerol, [4]-gingerdiol and hexahydrocurcumin.

Select concentrations of each antioxidant were added to a single-fold Argentinian lemon oil, which were then placed in a thermally accelerated storage chamber for four weeks. Upon removal, the lemon oils were analyzed by GC, measured for their peroxide values and tasted by an expert citrus panel in a high-acid tasting solution.

From the initial tasting, the two most preferred samples contained the antioxidants, tocopherols and tetrahydrocurcumin, which were also the two samples with the lowest peroxide values. Regarding the individual attributes, however, there weren't significant sensory differences between the oils; therefore, the tasting solutions of these oils were placed in the chamber for an additional two weeks. The sequential tasting revealed that hexahydrocurcumin was the most effective antioxidant in preserving the flavor quality of the lemon beverage. These findings prove that incorporating antioxidants can improve the flavor and stability lemon oil.

ACKNOWLEDGEMENT AND DEDICATION

I would like to acknowledge Rutgers University and International Flavors and Fragrances, Inc. (IFF) for supporting me through this entire endeavor, as well as, my advisors, Dr. Chi-Tang Ho and Dr. Henryk Daun, and my friends at IFF, Dr. Michael Chen, Dr. Sonia Liu, Dr. Hou Wu and Michael Hughes, who readily helped me in their area of expertise. I'd also like to thank my family and friends who have been tremendously supportive throughout the most arduous days; thank you for your care.

When I think of how I got here, the reflection is a bit hazy, but when I think about who got me here, the path becomes quite clear.

Mom: You were my first great influence; always giving your entire self to make life easier for your children. Thank you for the past thirty years of unconditional love, but most of all, thank you for your encouragement to always follow the *music of my heart*.

Dad: You define resilience. Thank you for teaching me to work hard while reminding me to enjoy the moments in between.

TV: Thank you for inspiring me and making me believe that, what I doubted was possible for myself, was truly attainable. You are the true definition of a mentor with whom I am so thankful for.

Lastly, to my "life coach" and to whom I dedicate this work to, my husband, Frank: I could not have done this without you... Your love let me soar.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

LIST OF APPENDICES

1. INTRODUCTION

1.1. The History and Background of Ginger

Ginger, botanically known as *Zingiber officinale* Roscoe, belongs to the Zingiberaceae family, which encompasses 47 genera and 1400 species, including turmeric (*Curcuma longa*) and cardamom (two main genera, *Elettaria* and *Amomum*.) The genus, *Zingiber,* contains 150 species; however, the only species extensively used for flavoring is *Z. officinale* (Ravindran and Nirmal Babu, 2005). It is grown from April to December at an optimal elevation between 300 and 900m (Pruthy, J.S., 1993); requiring a warm, humid climate while preferring light shade (Jayachandran *et al.,* 1991).

Ginger has been cultivated in southern Asian countries for over 3000 years and its discovery and value as a spice and medicinal plant has been well documented. Ginger has been mentioned in several places throughout history: "Round amongst them (the righteous in paradise) is passed vessels of silver and goblets made of glass… a cup, the admixture of which is ginger" (Koran 76: 15-17). One of the earliest references made was by Rabbi Benjamin Tudella from his travels between 1159 and 1173 A.D. who described the cultivation and trade of spices coming from the ancient port of Quilon, in the State of Kerala (Mahindru, 1982). The most significant event that changed the history of the spice trade was the landing of Vasco da Gama in 1498 on the west coast of India, Malabar Coast (Kerala) (Mahindru, 1982). Additional documentation dating back to 1298 A.D. was found in Marco Polo's travelogue stating that "good ginger grows here and is known by the name of Quilon ginger" (translation by Menon, 1929).

Since its discovery, India has been the largest producing country of ginger. Together, two of the states within the country, Kerala and Meghalaya, make up 30 to 40% of the world's total ginger production (FAOSTAT Database). The second largest ginger producer is Nigeria, followed by several other producers and exporters dispersed throughout the world: China, Jamaica, Taiwan, Sierra Leone, Fiji, Mauritius, Indonesia, Brazil, Costa Rica, Ghana, Japan, Malaysia, Bangladesh, Philippines, Sri Lanka, Solomon Islands, Thailand, Trinidad and Tobago, Uganda, Hawaii, Guatemala and many Pacific Ocean Islands (Ravindran and Nirmal Babu, 2005).

1.2. Traditional Uses of Ginger and Its Uses Today

1.2.1. As a Spice and Flavorant

Ginger is an ingredient found in the world's cuisine. Legend has it that the first gingerbread was made by a baker on the Isle of Rhodes near Greece around 2400 B.C. In the 1500's, gingerbread was known to be Queen Elizabeth I's favorite treat (Farrell, 1985) and during the Middle Ages, tavern keepers would keep a constant supply of ground ginger powder so customers could sprinkle it on their beer (Rosengarten, 1969). Today it is used in several products including Indian masala mixes, pumpkin pie spice, ginger ale, etc. Ginger based products are less popular in the Western world as compared to Australia, Thailand, Japan and China; the Buderim Company in Queensland, Australia, for example, produces more than 100 ginger-based products (Ravindran and Nirmal Babu, 2005).

1.2.2. For Medicinal Purposes

Ginger has been used for medicinal purposes long before its understanding. Traditionally ginger is used in both fresh and dried forms in Chinese, Indian, Indonesian and Japanese medicines for the treatment of: arthritis, rheumatism, sprains, muscular aches, asthma, sore throats, motion sickness, indigestion, nausea, vomiting, diarrhea, constipation, hypertension, dementia, etc. (Cho *et al.,* 2001; Badreldin *et al.,* 2008; Pharmacopoeia of the People's Republic of China, 2010). In ancient India, ginger was primarily used as a medicine rather than as a flavorant and was referred to as the *mahaoushadha* (the great medicine) and *vishwabheshaja* (the universal cure) (Ravindran and Nirmal Babu, 2005). Ayurveda is a traditional approach to medicine that is native to India; ginger has been widely used in Ayurvedic medicine to treat a variety of gastrointestinal ailments. Modern homeopathic uses of ginger are quite similar and are popularly used for the treatment of indigestion, nausea due to motion sickness, pregnancy and for patients undergoing chemotherapy.

1.3. The Chemical Components of Ginger

Steam distillation and supercritical carbon dioxide (CO_2) extraction yield an essential oil containing volatile components, whereas, solvent extraction yields oleoresins containing non-volatiles and tastants (Ravindran and Nirmal Babu, 2005). The first chemical study of ginger (Cochin) was done by J.O. Thresh in 1879 (Yearbook of Pharmacy, 1879, 1881 and 1882).

Some of the main volatiles identified by Connell in 1970 are "the

sesquiterpene hydrocarbons: (-)-α-zingiberene, (+)-ar-curcumene, β-bisabolene, β-sesquiphellandrene, farnesene, γ-selinene, β-elemene and β-zingiberene. Other monoterpene hydrocarbons identified are: α-pinene, β-pinene, myrcene, β-phellandrene, limonene, *para*-cymene, cumene, and oxygenated compounds: 1,8-cineole, d-borneol, linalool, neral, geranial, bornyl acetate; aliphatic aldehydes: nonanal, decanal; ketones: methylheptenone; alcohols: 2-heptanol, 2-nonanol; esters of acetic and caprylic acid and chavicol" (Connell, 1970).

The non-volatiles known to be responsible for the pungency of ginger are the gingerols and shogaols. [6]-gingerol was first identified by Lapworth in 1917; in 1969, Connell and Sutherland established the *S*-configuration for the hydroxyl group (Figure 1). Gingerols undergo dehydration readily due to the thermally labile beta-hydroxy-keto group, thereby forming the corresponding shogaols (Figure 2).

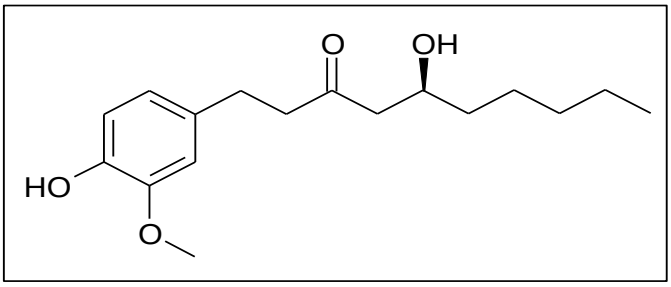


Figure 1. *S*-[6]-gingerol

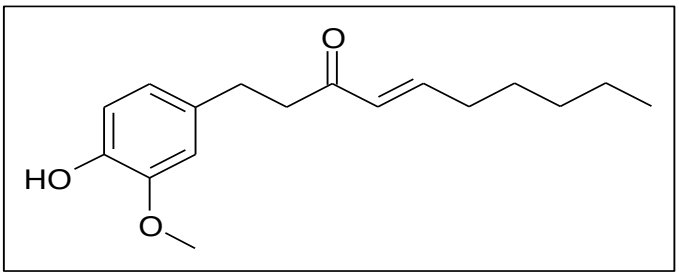


Figure 2. [6]-shogaol

The production of *n*-hexanal after alkaline hydrolysis of gingerol afforded the name [6]-gingerol while the name shogaol, was derived from the Japanese word for ginger, “shoga”. Gingerols and shogaols are not only responsible for the pungency of the ginger oleoresin but have been proven to be responsible for its antioxidant capability as well (Kikuzaki and Nakatani, 1993).

1.4. The Use of Ginger as an Antioxidant

1.4.1. Health Related Active Compounds

Antioxidants are present in nutraceuticals which refers to foods that have inherent health benefits greater than their dietary need and are consumed to help treat or prevent specific diseases. Nutraceuticals are typically plants, fruits, vegetables, roots and seeds because they internally produce their own antioxidants to combat oxidative stress offering a source of natural antioxidants. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols are some of the natural antioxidants produced by the before mentioned botanicals for their own oxidative protection (Ghasemzadeh *et al.,* 2010).

Oxidative damage in living tissue results in an inflammatory response which can also lead to the increased risk of chronic diseases such as cancer, coronary atherosclerosis and other age-related, degenerative diseases (Stoilova *et al.,* 2007; Astley, 2003). Dietary antioxidants found in nutraceuticals help to eliminate or prevent the accumulation of the damaging oxidative products within the botanical and have proven to be useful for human health as well.

Gingerols and shogaols are the most well-known and studied antioxidants in

ginger and have shown to have several pharmacological effects including the inhibition of prostaglandin biosynthesis, anti-hepatotoxicity, cardiotonic and anti-platelet (Cho *et al,* 2001).

1.5. The Use of Antioxidants in Foods and Flavors

Similarly to the auto-oxidation of lipids in biological membranes, oxidation degradation can also occur in food. Fat oxidation, for example, leads to an occurrence of several chain reactions forming double bonds, alcohols, aldehydes and ketones which generate off-flavors and the reduce the nutritional value (Stoilova *et al*., 2007).

For decades, food technologists and flavorists around the world have used antioxidants to retard or inhibit the spoilage and rancidity of foods caused by oxidation reactions. The most common synthetic antioxidants used in the food and flavor industry are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). However, as the trend amongst food consumers and suppliers to avoid synthetic additives continues to increase, so does the importance of conducting research that identifies, understands and utilizes antioxidants of natural origin.

The efficiency of ginger extracts, and that of gingerols and shogaols, has been compared to the synthetic antioxidants, BHT and BHA, and select natural antioxidants, namely, α-tocopherol and ascorbic acid, which will be discussed in the literature review section of this composition.

2. LITERATURE REVIEW

2.1. Ginger as an Antioxidant

2.1.1. Antioxidant Studies of Ginger Extracts

The efficiency of ginger extracts have been compared to that of the synthetic antioxidants, BHT and BHA, and the natural antioxidant, α-tocopherol. For example, the acetone extracts of the rhizomes of two ginger species, *Z. cassumunar* and *Z. officinale*, were proven to be comparable and in some cases, stronger antioxidants in the inhibition of lipid peroxidation by ferric thiocyanate (FTC) and thiobarbituric acid reactive species (TBARS) methods. In this study, *Z. officinale* was a stronger antioxidant than α-tocopherol and comparable to that of BHT (Jitoe *et al.,* 1992; Kikuzaki and Nakatani, 1993).

CO_2 ginger extracts, which are rich in polyphenols, are known to donate their hydrogen atoms to reduce free radicals. In a study by Stoilova *et al.* (2007), the efficacy of the CO_2 extract of Z*. officinale* was compared to BHT by measuring the ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and inhibit the oxidation of linoleic acid. The ginger extract had a significantly higher scavenging ability regarding DPPH and in the study of lipid oxidation, the ginger extract was better at both, inhibiting the formation of conjugated dienes, as well as, inhibiting the oxidation of linoleic acid.

Lastly, a multi-solvent extraction of ginger was compared to BHA and BHT (both at 200ppm) during a six month storage of sunflower oil. Here the peroxide and free fatty acid values were documented and it was found that the ginger extract

was more effective than BHA at 1600 and 2400ppm and comparable to BHT at 2400ppm (Salariya and Habib, 2003).

2.1.2. Comparative Antioxidant Studies of Active Compounds Found in Ginger

Several conclusions have been made regarding the structure-activity relationship amongst the gingerol and shogaol analogues. Dugasani *et al*. (2010) showed that in the scavenging of DPPH, superoxide and hydroxyl radicals, the pattern of effectiveness is as follows: [6]-shogaol > [10]-gingerol > [8]-gingerol> [6]-gingerol, which implies that increasing the carbon chain length will increase the efficacy. However, studies done by Kikuzaki and Nakatani (1993), Masuda *et al*. (2004) and Cho, K. *et al*. (2001) challenge this correlation.

Kikuzaki and Nakatani (1993) studied the structure activity relationships of 12 isolated antioxidants from a dichloromethane extract and compared them to that of α-tocopherol. They used the FTC and TBARS methods with linoleic acid as the substrate and concluded that the increase in antioxidant activity was due to the constituents along the carbon chain and the substitution patterns on the benzene ring. In both classes of the gingerol-related structures and diarylheptanoids (Figure 3), the diacetate moieties were the strongest antioxidants. In comparing the stereoisomers having the configuration of *3R,5S* versus the *3S,5S* configuration, the *3R,5S* moiety was also stronger. Overall, however, all 12 isolates were more effective than α-tocopherol.

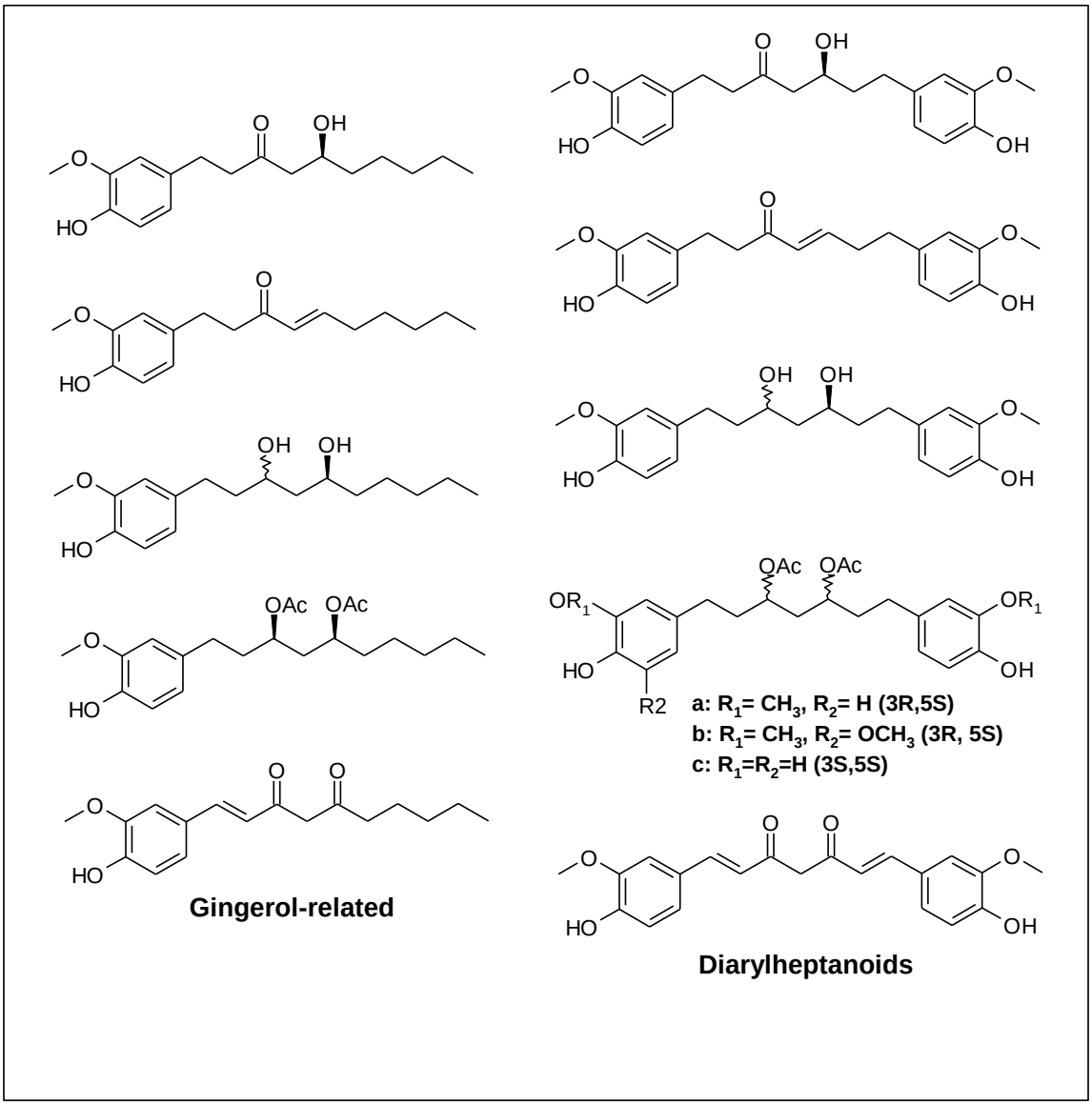


Figure 3. Isolated compounds from ginger

Masuda *et al.* (2004) reported testing the isolated gingerol-related compounds and diarylheptanoids for their DPPH radical scavenging activity and the inhibitory effect on the oxidation of methyl linoleate under aeration and heating using the Oil Stability Index (OSI) method. In this study, no significant differences in activity amongst the compounds of different alkyl chain lengths, in neither DPPH

nor OSI methods, were observed. However, there were differences between the efficacy of the dehydrogingerdiones and the gingerols in that, dehydrogingerdiones were weaker antioxidants in scavenging DPPH but more effective in retarding the auto-oxidation of the oil. These results again, support the inferences that the substrates and substituents on the alkyl chain contribute to the antioxidant efficacy more so than the alkyl chain length.

Lastly to contradict the correlation of increasing antioxidant strength with chain length, Cho *et al*. (2001) isolated [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol from a methanol and subsequent ethyl acetate extraction. The antioxidant strength of each isolate was tested against BHT and BHA in scavenging DPPH. It was reported that [4]-gingerol and [6]-gingerol were superior to BHT but [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol were inferior to BHA.

Zancan *et al*. (2002) studied different extraction techniques along with CO_2 and solvent combined CO_2 extractions. They compared the chemical compositions of each, containing varying quantities of gingerols and shogaols, and determined that the extractions with higher amounts of gingerols and shogaols had a higher antioxidant response during the coupled oxidation of linoleic acid and β-carotene. They also confirmed that the gingerol and shogaol-related moieties were responsible for the increased efficacy and indicated that using co-solvents, such as, ethanol or isopropanol is advisable because it assisted the CO_2 in extracting greater amounts of gingerols and shogaols.

2.2. Antioxidants in Food Applications

Oxidative degradation reactions inherent in food not only shorten shelf-life but also make food products unacceptable for the human palette. Pre-cooked, ground meats are prone to lipid oxidation especially those with a high fat content of 20-30%. To extend shelf-life, it has become a common practice to incorporate antioxidants into meat products (Biswas *et al*., 2001) and in response to the demand for *all-natural* label claims, natural extracts with known antioxidant activity have been studied. In a six-month study by Sasse *et al*. (2009), grape seed extract, rosemary oleoresin and a water-soluble oregano extract were compared to a few commonly used synthetic antioxidants. They were tested in pre-cooked and subsequently frozen pork patties, and the effectiveness of the antioxidants on the oxidative stability measured by TBARS, was as follows: propyl gallate > grape seed> BHA> BHT> rosemary oleoresin> oregano water soluble extract. This indicates that natural extracts may be used in place of synthetic antioxidants in several food applications.

2.2.1. The Use of Ginger as an Antioxidant in Food

An ethanolic ginger extract made from ground ginger rhizomes was mixed with raw, ground pork meat (0.5% w/w) with which, patties were formed. The patties were roasted and kept frozen at 4°C for 21 days before the fat was extracted. Compared to the patties without ginger extract, those with, had lower evidence of triacylglycerol hydrolysis, hydroperoxide formation and overall, lower peroxide values (Takacsova *et al.,* 2000).

Another application involving the antioxidant effect of ginger powder has been achieved in cookie dough. Both ginger and cumin powder were tested for their antioxidant ability in scavenging DPPH using the methanolic extracts of the finely ground cookies containing one to five percent powder based on 100g of flour. The results showed a linear increase in the antioxidant activity with the increased percentage of both powders, and ginger, having a greater scavenging effect than cumin (Abdel-Samie *et al*., 2010). The linear increase in efficiency is also congruent with the study of ginger extract in sunflower oil and the thermal stability and antioxidant strength after heat treatment of the ginger extract (Salariya and Habib, 2003).

2.3. Citrus Instability

Citrus flavors are quite popular amongst the general population as a flavor preference; however, the main constituents, in namely lemon oil, limonene and citral, can be rather unstable. This instability results in the loss of quality and consumer acceptability and therefore, shelf-life is limited.

2.3.1. Limonene Oxidation

Limonene (4-isopropenyl-1-methyl-cyclohexene) is a monocyclic terpene and the major constituent of citrus essential oils making up over 95% of lemon peel oil (Djordjevic *et al.,* 2007). It is a chiral molecule in which the *R*-enantiomer exists in nature as D-limonene ((+)-limonene) (Figure 4) and can be obtained by the steam distillation of citrus fruits.

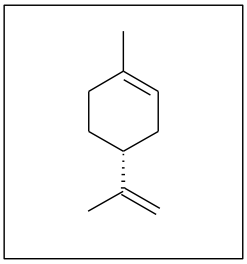


Figure 4. D-limonene

Although limonene is relatively stable and can be distilled without decomposition, at elevated temperatures around 300°C, it will racemize forming isoprene which easily oxidizes in moist air (Karlberg *et al*., 1992). The flavor of limonene is fresh, green, citrusy and slightly piney, while the oxidation product, limonene 1,2-epoxide, is greener, with weedy, minty and herbaceous notes.

In the presence of acid, a hydrogen shift may occur forming a carbocation; once this happens, limonene is racemized and the chirality of all degradation products is lost. The carbocations that are formed can lead to addition products, such as, α-terpineol, β-terpineol and γ-terpineol, while the dehydration reactions of limonene can produce *p*-cymene (Thomas and Bessiere, 1989).

McGraw *et al*. (1999) studied the thermal degradation of limonene in the presence of oxygen and found that the process can be divided into several types of oxidation pathways. The first is the dehydrogenation of a six-membered ring containing one or two double bonds and in the case of limonene, thymol is produced (Figure 5a). The second is the formation of epoxides which can produce limonene oxide (Figure 5b) and the last to mention involves the reaction of singlet oxygen which will lead to the oxidation of the carbon adjacent to the carbon-carbon double bond, known as allylic oxidation (McGraw *et al*., 1999).

Since 1914, it has been known that limonene is very sensitive to oxygen and it was observed that in a drum of limonene under the Australian sun, perillyl alcohol (Figure 5c) was formed. As with the oxidation of unsaturated fatty acids, limonene oxidation initially results in the formation of hydroperoxides which can then undergo scission reactions leading to the formation of numerous products including perillyl acetate, carveol, carveol acetate and carvone (Figure 5d) (Djordjevic *et al.,* 2007). Degradation products of monoterpenes and their derivatives can also be traced back to the rearrangement of the skeletal structure; for example, one rearrangement product of limonene is eucarvone (Figure 5e) (McGraw *et al*., 1999).

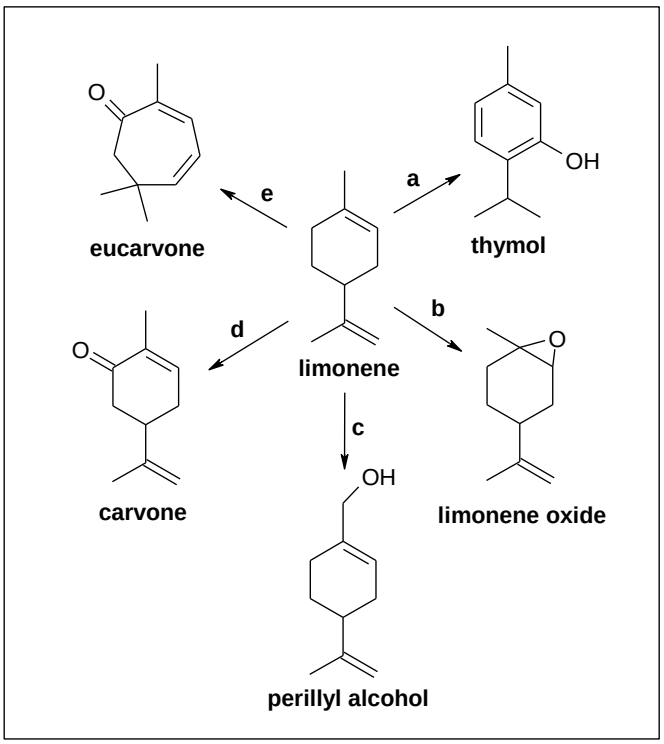


Figure 5. Reaction products of limonene

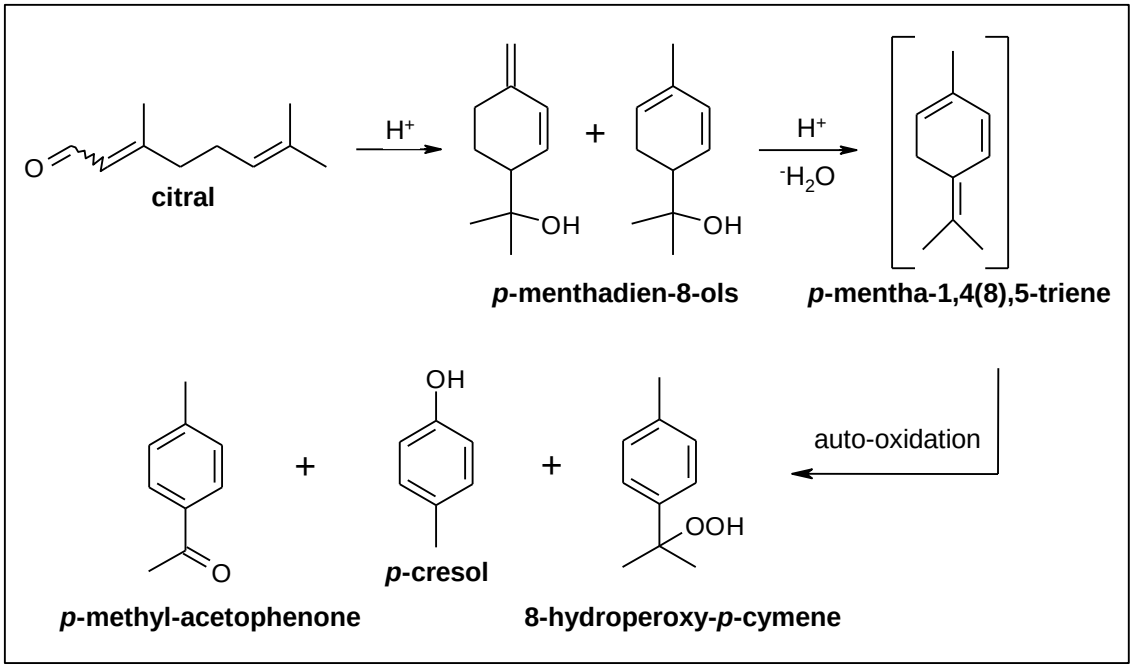
Syntheses using limonene as a starting reagent have been vastly studied. As early as, 1922, polymers were formed by reacting limonene with phenols and the

addition of hydrogen sulfide with limonene forms menth-1-ene-8-thiol, which is commonly known in the flavor industry as, *grapefruit mercaptan* (Thomas and Bessiere, 1989).

2.3.2. Citral Degradation

Citral (3,7-dimethyl-2,6-octadienal) is an unsaturated monoterpene aldehyde with an additional α,β-unsaturated double bond and is comprised of two geometric isomers, neral and geranial, in the ratio of 2:3 (Liang *et al.,* 2004). Citral is the characterizing constituent in lemon oil lending a fresh and zesty aroma; however, it is known to degrade rapidly under low pH and oxidative stress conditions, yielding undesirable off-flavors.

The acid-catalyzed cyclization of citral begins with the isomerization of geranial to neral, which can form monoterpene alcohols, such as, *p*-menthandien-8-ol (Scheme 1). After further oxidation, *p*-cymene-8-ol can be formed, while dehydration reactions will produce aromatic compounds, such as, α,*p*-dimethyl-styrene, *p*-cymene and *p*-cresol (Djordjevic *et al*., 2007; Ueno *et al*., 2006). *p*-Cresol and *p*-methylacetophenone are known to be the most offensive off-flavors of citral degradation (Djordjevic *et al*., 2007).



Scheme 1. Proposed mechanism for the formation of *p*-cresol from citral (Ueno *et al*., 2006)

2.3.3. Stability Efforts

Several attempts have been made to inhibit limonene from oxidizing and citral from degrading. These efforts include: reducing storage temperature, adjusting pH, modifying headspace, storage in dark containers and using antioxidants within the system itself.

Karlberg *et al.* (1994) added BHT to an open container (exposed to air) of limonene at room temperature and found that when the BHT was consumed, the auto-oxidation took place similarly to the samples without having any antioxidant added. However, when the sample was kept in a closed container in the dark at 4°C, the concentration of limonene remained stable for the duration of the 12 month study.

In another effort to stabilize limonene, oleoresins, such as, rosemary (*Rosmarinus officinalis* L.), anise (*Pinpinella anisum* L.), caraway (*Carum carvi* L.) and dill (*Anethum graveolens* L.) were compared to the commonly used antioxidants: mixed tocopherols (50%), BHA and BHA/BHT (1:1) by measuring the peroxide values and monitoring the degradation markers (limonene epoxides, carveols and carvone) on the GC. Out of the oleoresins, rosemary was the most effective in inhibiting limonene oxidation; it was more effective than BHA, comparable to the mixed tocopherols and slightly less effective than the combined BHA/BHT (Lee and Widmer, 1994).

Kimura *et al*. (1983) used common antioxidants, such as, BHT, BHA, *n*-propyl gallate, α-tocopherol, nordihydroguaiaretic acid and *n*-tritriacontan-16,18-dione, in a citric acid solution of citral but found that none of these antioxidants were able to inhibit citral from degrading. Peacock and Kuneman (1985), however, found that the use of isoascorbic acid inhibited the formation of *p*-cymene-8-ol and its dehydration product, α,*p*-dimethylstyrene. Liang *et al.* (2004) found that natural antioxidants (grape seed, pomegranate seed, green tea and black tea extracts) inhibited the formation of *p*-methylacetophenone by blocking the pathway of *p*-cymene-8-ol and lastly, is the combined use of natural antioxidants in emulsion systems. In the study by Yang *et al*. (2011), the emulsion systems employing either β-carotene or tanshinone, were effective in diminishing the rate of citral degradation.

3. HYPOTHESIS

The antioxidants found in ginger will enhance the flavor and stability of lemon oil.

4. RESEARCH OBJECTIVES

4.1 Ginger Selection

To investigate the nine ginger powders, extraction methods, extraction solvents and antioxidant activity to determine which ginger powder should be selected for further, in-depth work.

4.2 Fractionation and ORAC Monitoring

Once the ginger powder is selected, the appropriate extraction protocol must be followed for its use in the SepBox®. Each fraction collected from the SepBox® will be tested for its ORAC value to determine which fractions are responsible for the antioxidant efficacy of the ginger extract.

4.3 Identification of Strongest Antioxidants

Select ginger fractions will be identified based on their ORAC values and the quantity available. This will be done using the developed LC/MS/UV method, MS/MS, HRMS, as well as, referencing the literature (Jiang *et al*., 2007).

4.4 Use of Antioxidants in Lemon Oil

Select concentrations of each antioxidant will be employed into a single-fold Argentinian lemon oil which will be placed in the thermal acceleration storage chamber for four weeks. Upon removal, the lemon oils will be analyzed by GC, measured for their peroxide values and tasted by an expert citrus panel in a high-acid tasting solution. The oils with antioxidants will be compared to two controls

without antioxidants: one refrigerated and one thermally accelerated sample, to determine how effective the antioxidants are at preventing the lemon oil from degrading.

5. EXPERIMENTAL

5.1. Materials

5.1.1. Ginger Powders

Synthite Industries Limited (Kerala, India) provided the following: Synthite Rajakumari, Synthite Irutti, Synthite Nigerian and Synthite Shimoga. *Whole Herb Company* (California, USA) provided large quantities of both Whole Herb Nigerian and Whole Herb India ginger powders and *Buderim Ginger America, Inc* (New Jersey, USA) provided both Buderim Australian and Buderim Fijian ginger powders. PharmaChem Chinese ginger was purchased from *PharmaChem Laboratories* (New Jersey, USA.)

5.1.2. Solvents, Reagents and Supplies

Acetone (Fisher A929-4, Acetone Optima®); hexane (Fisher H302-4 HPLC Grade); methanol (Fisher A452-4 HPLC Grade); water (Fisher W5-4 HPLC Grade); dichloromethane (Fisher D150-4 HPLC Grade). Acetone/acetonitrile/methanol (1:1:1 by volume) and formic acid were purchased from EMD Chemicals, Inc. (Pennsylvania, USA). The ethanol (HPLC Grade) was purchased from Pharmco-Aaper (Connecticut/ Kentucky, USA). DPPH and hexahydrocurcumin were purchased from Sigma-Aldrich (Missouri, USA). Mixed tocopherols (50% in sunflower oil) was purchased from VitaBlend Nederland B.V. (Netherlands). [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, [8]-shogaol and [10]-shogaol were purchased from ChromaDex (California, USA). Tetrahydrocurcumin (TetraPure®) was from Sabinsa Corporation (New Jersey, USA). [4]-Gingerol, [4]-gingerdiol, [4]-shogaol, [6]-gingerdiol and octahydrocurcumin were synthesized by IFF R&D (New

Jersey, USA). OxiSelect Oxygen Radical Antioxidant Capacity (ORAC) Activity Assay was purchased from Cell BioLabs, Inc. (California, USA). C4-Silica from Macherey-Nagel GnmH & Co. (Berlin, Germany). Single-fold Argentinian-type lemon oil was purchased from Capua (Calabria, Italy). For the peroxide assay, the chloroform ($CHCl_3$, C606SK, HPLC Grade), glacial acetic acid (A35), potassium iodide (KI, P412) and 0.1 N solution of sodium thiosulfate (12427-001, Acros) were purchased from Fisher Scientific (Pennsylvania, USA); the starch indicator (8050-16) was from Ricca Chemical Company (Texas, USA). For the high-acid tasting solution, sodium benzoate and citric acid were purchased from Mitsubishi International (New Jersey, USA) and the high-fructose corn syrup was purchased from Paulaur Corporation (New Jersey, USA).

5.2. Instruments and Equipment

Mettler Toledo Model: HG63 Halogen was used to measure moisture content. *Orbital Shaker ISF-1-V; Adolf Kuhner AG, Schwez* was used for the acetone extractions of ginger powders. *Buchi Speed Extractor E-914* (Switzerland) was used for the hexane extractions of ginger powders. Rotary Evaporator by *Buchi: Rotavapor R-210* and *Vacuum Controller V-850* (Switzerland) were used for solvent removal. *Agilent 6890 Gas Chromatograph* equipped with a *5973 Mass Selective Detector* was used for GC profiling of acetone extracts and for peak identification of markers in lemon oils; *Agilent 7890A* was used for the area percent of the identified markers in the lemon oils. The *Agilent 1200* was used for HPLC profiling of ginger extracts and the DPPH assay with a Luna C18 column by *Phenomenex*. For structure

identification, the LC/MS/UV Thermo LSQ Mass was used and the High Resolution Mass Spectrometer (HRMS) *Thermo LTQ Orbitrap* was used to obtain exact mass. The percolator was made for IFF by *ChemGlass*. *Beckman Coulter Spectrometer DTX880* was used to measure the fluorescence during the ORAC assay employing a 480nm excitation filter and a 520nm emission filter. *SepBox® 2D-5000 by sepiatec* (Berlin, Germany) utilizing *MAC* Process traps and columns (including Carbo, C4, C8 and C18.) *Branson 3150 Sonicator*; *Thermo Scientific Centrifuge CL10*; *IsoTemp Vacuum Oven Model 285A* (Fisher Scientific); *Biotage SP1* utilizing column KP-Sil 40+M; *Spectroline Model CC-80 Ultraviolet Fluorescence Analysis Cabinet* (Spectronics Corporation) with a short wave UV of 254 nm and a long wave UV of 365 nm. Thermally Accelerated Storage Chamber "*Hot Box"* (Scientific Climate Systems, Inc.)

5.3. Methods and Protocols

5.3.1. Moisture Content

All moisture contents were taken in triplicate and averaged.

5.3.2. Extraction of Ginger Powders and Fractionation of Synthite Nigerian Acetone Extract

5.3.2.1. Acetone Extracts Using Orbital Shaker

Each ginger powder (30 g) was loaded into an orbital shaker flask with acetone (270 g) and covered with aluminum foil. The samples were agitated at 150 revolutions per minute (rpm) for 72 hours at 21°C. The samples were then

decanted, filtered and the solvent was removed under mild heat (40°C) and reduced pressure (200 mmHg).

5.3.2.2. Hexane Extracts Using Speed Extractor

Using the Speed Extractor, solvents with increasing polarity, in the order of hexanes, methanol then water, were run through the ginger powder, under a set pressure (140 bar for hexane and methanol, and 40 bar for water) and a temperature of 60°C, 75°C and 96°C, respectively. The samples were decanted, filtered and the solvent was removed under mild heat (40°C) and reduced pressure (200 mmHg).

5.3.2.3. Percolator Extract for SepBox®

600 g of Synthite Nigerian ginger powder was loaded into the percolator and 2800 mL of hexane was added. The mixture was circulated with a pump for three hours at 40°C (the temperature of the heating oil in the jacket was 52°C). The solvent was drained and concentrated to a residue, using the rotary evaporator under reduced pressure (temperature: 40°C; pressure: 200 mmHg) and labeled "Synthite Nigerian Hexane Extract." Yield: 22.9 g. To the spent ginger powder, 2800 mL of acetone was added into the percolator and circulated with a pump for another three hours at 40°C (the temperature of the heating oil in the jacket was 52°C). The solvent was drained and the procedure was repeated using another 2800 mL of acetone. The two extracts were combined and concentrated to a residue, using a rotary evaporator under reduced pressure (temperature: 40°C; pressure: 200

mmHg) and labeled “Synthite Nigerian Acetone Extract.” Yield: 24.2 g and the HPLC can be found in the appendix (Appendix 23b). Lastly, 2800 mL of ethanol was added to the percolator to the twice spent ginger powder and circulated with a pump at 60°C for five hours. The solvent was drained and concentrated to a residue, using a rotary evaporator under reduced pressure (temperature: 40°C; pressure: 200 mmHg) and labeled “Synthite Nigerian Ethanol Extract.” Yield: 15 g.

5.3.2.4. SepBox® Fractionation

5.033 g of Synthite Nigerian acetone extract was added to 40 mL of methanol with the assistance of sonication for 30 minutes. The dilution was centrifuged for five minutes at 3000 rpm, decanted and 20 mL of acetone was added to the residue. The acetone dilution was centrifuged, combined with the methanol solution and filtered using a 1 μm syringe filter followed by a 0.22 μm syringe filter. After, the ginger methanol/ acetone solution was placed in a round bottom flask and 29.68 g of C4-silica was added; the system was sonicated and put onto the rotary evaporator to remove the solvent at a mild temperature (40°C) and reduced pressure (200 mmHg).

Approximately 30 g of ginger sample on silica was added to the injection column containing a 7 g layer of C4-silica and topped off with another 7 g of C4-silica. The injection column was flushed with water to remove any water soluble sugars, starches, etc., present in sample which directly exited the system and went into the fraction collector. The sample remaining on the column then eluted to the

main separation column in which a series of trapping and separation began; following a detailed IFF developed protocol for fractionating natural extracts.

5.3.3. Analytical Work on Ginger Extracts and Lemon Oils

5.3.3.1. Gas Chromatogram (GC) Work on Ginger Acetone Extracts

From the orbital shaker acetone extracts, 10% dilutions in acetone filtered and injected into the GC. The instrument method called for a 1 μl injection, a split ratio of 50:1, with a 250°C inlet temperature, an OV1 column (50 m x 0.23 mm x 0.5 μm), a carrier flow (He) of 1.0 ml/min and an oven temperature program of 40°C ramped at 2°C per minute to 310°C with a 40 minute hold.

5.3.3.2. Gas Chromatogram/Mass Spectrometry (GC/MS) Work on Lemon Oils

The GC method called for a 1 μl injection of the neat oil, a split ratio of 100:1, with a 70°C inlet temperature, an OV1 column (60 m x 0.25 mm x 1 μm), a carrier flow (He) of 2.0 ml/min and an oven temperature program of 70°C ramped at 3°C per minute to 220°C with a 15 minute hold.

The MS parameters were as follows: 1 μl injection of neat lemon oil, a split ratio of 30:1, 70°C inlet temperature, an OV1 column (30 m x 0.25 mm x 0.25 μm), a carrier flow (He) of 1.5 ml/min and an oven temperature program of 70°C ramped at 5°C per minute to 250°C with a 10 minute hold.

5.3.3.3. High Performance Liquid Chromatography (HPLC) Profiling

The HPLC profiling of each acetone and hexane extract was done using 10% dilutions in acetone. The neat hexane extracts were placed into the vacuum oven at 40°C and 0.3 mmHg overnight before diluting with acetone.

LC Conditions:
Column: Luna C 18 (250 x 4.6 mm), 5 μm
Eluent: A= 0.1% formic acid in H_20; B = 0.1% formic acid in ACN
Gradient:

Time	%A	%B	Flow (mL/min)
0.00	90.0	10.0	0.7
30.00	20.0	80.0	0.7
35.00	0.0	100.0	07
36.00	90.0	10.0	0.7
40.00	90.0	10.0	0.7

Injection Volume: 10 μl
UV λ: 280 nm
Solvent for the samples: Acetone

5.3.3.4. Structure Identification of Major Components in Select Ginger Fractions

A developed LC/UV/ MS method was used for the analysis of the samples and based on the HRMS, literature and MS^n patterns, several compounds were tentatively proposed. All the samples were diluted with or dissolved in acetone to a concentration of 0.5%. The solutions were filtered prior to LC/MS analysis.

LC Conditions:
Column: Luna C 18 (250 x 4.6 mm), 5 μm
Eluent: A= 0.1% formic acid in H_20; B = 0.1% formic acid in ACN
Gradient:

Time	%A	%B	Flow (mL/min)
0.00	90.0	10.0	0.7
30.00	20.0	80.0	0.7
35.00	0.0	100.0	07
36.00	90.0	10.0	0.7
40.00	90.0	10.0	0.7

Injection Volume: 10 μl
Solvent for the samples: Acetone

MS conditions:
Ion Mode: APCI positive
Capillary Voltage: 14 V
Capillary Temperature: 225 ^{o}C
Discharge Current: 5 μA
Sheath Gas Flow: 65 arb
Isolation Width: 1.5 amu
Normalized Collision Energy: 35%

5.4. Antioxidant Assays

5.4.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Activity

0.05% dilutions of ginger extracts in methanol were made for each extract. 0.5 mL of the 0.05% ginger extract in methanol was added to 0.5 mL of a 0.1% dilution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. These were compared to the blank (0.5 mL of the 0.1% DPPH dilution in methanol with 0.5 mL methanol) to serve as a reference. Three hours later, the absorbance was measured at 517 nm using the LC and the antioxidant activity were measured using the calculation below.

Antioxidant Activity (%) = (1-abs of sample/ abs of DPPH ref)*100

Surveyor LC Conditions:
Eluent: A= 0.1% formic acid in H_20; B = 0.1% formic acid in ACN
Gradient:

Time	%A	%B	Flow (mL/min)
0.00	10	90	0.7
1.00	10	90	0.7

Temperature: ambient
Detection: UV @ 517 nm
Injection Volume: 10 μl
Solvent for the samples: Methanol

5.4.2. Oxygen Radical Absorbance Capacity (ORAC)

All 18 ginger extracts (hexane extracts from speed extractor and acetone extracts from orbital shaker) and the 316 fractions from the hexane washed acetone extract of Synthite Nigerian, were tested for their ORAC values at 0.01%; following the protocol provided by Cell Bio Labs.

5.4.3. Peroxide Value Assay

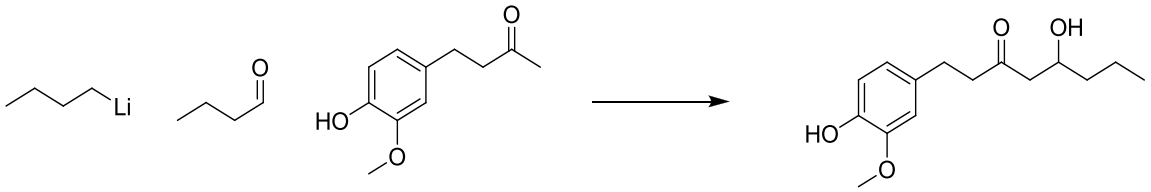
Following the protocol developed by IFF, approximately 5 g of oil is weighed into a 250 mL Erlenmeyer flask. 30 mL of an acetic acid-chloroform solution (3:2 v/v) is added, followed by the addition of 0.5 mL of saturated potassium-iodide solution and agitation for one minute. With vigorous agitation, 30 mL of distilled water is added followed by 0.5 mL of starch indicator. The flask is subsequently titrated with a 0.1 N sodium thiosulfate solution. The end-point is reached when the blue color disappears and a yellow color remains for 30 seconds. The peroxide values can be calculated using the equation below.

$$\textbf{Peroxide Value} = \frac{(S - B)\,(N)\,(1000)}{\text{Weight of Oil}}$$

S: Titration Volume of the Sample
B: Titration Volume of the Blank
N: Normality of the Sodium Thiosulfate solution

5.5. Syntheses of Ginger-related Compounds

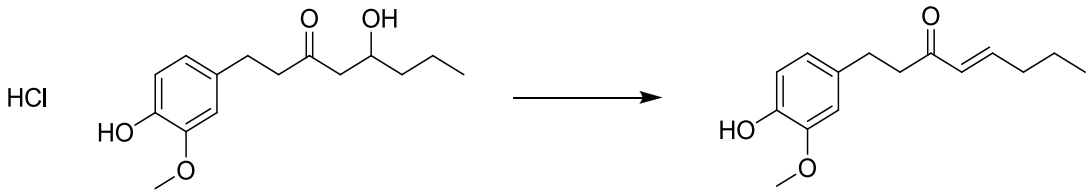
5.5.1. [4]-Gingerol



Dissolve 4.0 g of zingerone in 100 mL of dry tetrahydrofuran (THF) and cool the mixture with a dry ice bath to -78˚C. Under nitrogen (N_2), add 8.5 mL of 2.5 N butyl lithium solution (dropwise) to the reaction mixture. Stir for 30 minutes at -78 ˚C after the addition, then add 10.0 mL of 2.0 N lithium diisopropylamide (LDA) solution (dropwise) to the mixture. Stir the mixture at -78 ˚C for 3h, then add a solution of 1.5 g butyraldehyde in 20 mL of dry THF (dropwise). Stir the mixture at -78˚C for 3h, remove the dry ice bath and stir at 0˚C for 1h. Quench the reaction by slowly adding 100 mL of 1N hydrogen chloride (HCl). Extract the mixture with 200 mL of ether, then wash the organic layer with brine twice, and dry with magnesium sulfate ($MgSO_4$).

After filtration and concentration, the crude was purified by flash chromatography (silica gel, ethyl acetate (EtOAc)/Hexanes). 2.5 g of product was obtained and the yield was 51%. 400-MHz ^1H NMR ($CDCl_3$) δ: 6.82 ppm (d, 1H, J = 7.92 Hz), 6.62-6.71 ppm (m, 2H), 5.51-5.55 (br, 1H), 4.00-4.10 (m, 1H), 3.87 ppm (s, 3H), 2.45-2.95 (m, 7H), 1.30-1.52 ppm (m, 4H), 0.92 ppm (t, 3H, J = 7.02 Hz).

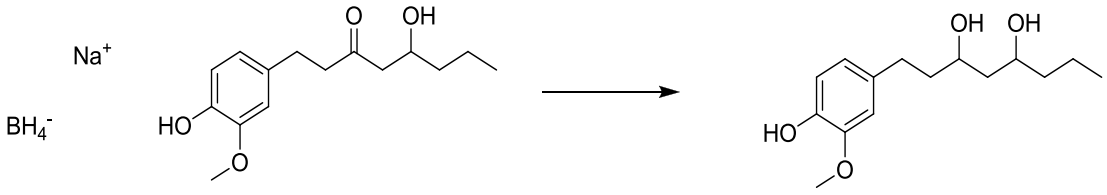
5.5.2. [4]-Shogaol



Dissolve 3 g of 4-gingerol in 150 ml of THF, add 150 ml of 10% HCl solution to the flask and reflux for 4 h. Cool down and extract with 200 ml of ether, wash the organic layer with brine twice, then dry with $MgSO_4$.

The crude was purified by flash chromatography (silica gel, EtOAc/Hexanes) in which 1.8 g of product is obtained (Yield: 64.4%). 400-MHz ^1H NMR ($CDCl_3$) δ: 6.65-6.85 ppm (m, 4H), 6.09 ppm (d, 1H, J = 15.89 Hz, of t, J = 1.50 Hz), 5.61 ppm (s, 1H), 3.86 ppm (s, 3H), 2.80-2.90 ppm (m, 4H), 2.17 ppm (t, 2H, J = 7.11 Hz, of d, J = 1.50 Hz), 1.43-1.53 ppm (m, 2H), 0.93 ppm (t, 3H, J = 7.45 Hz).

5.5.3. [4]-Gingerdiol

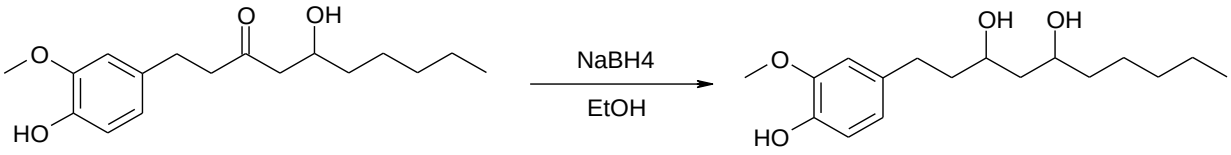


Dissolve 0.4 g of sodium borohydride ($NaBH_4$) in 20 ml of ethanol, then cool down with an ice bath. Add the solution of 2.5 g [4]-gingerol in 10 mL of ethanol (dropwise) to the mixture and keep the temperature around 0˚C. After addition, remove cooling bath and stir at room temperature for 2 h. Cool down again to 0˚C, add 10 mL of 1 N HCl (dropwise) to decompose any excess of $NaBH_4$. Transfer the

mixture to a separatory funnel, add 100 ml of brine, and extract with 200 ml of EtOAc. Wash the organic layer with brine twice and dry with $MgSO_4$.

The crude was purified by flash chromatography (silica gel, EtOAc/Hexanes) in which, 2.0 g of product is obtained (Yield: 79%). 400-MHz ^1H NMR ($CDCl_3$) δ: 6.82 ppm (d, 1H, J = 7.92 Hz), 6.62-6.71 ppm (m, 2H), 5.54 ppm (s, 1H), 3.87 ppm (s, 3H), 3.60-4.40 (br, m, 2H), 2.50-3.20 (m, 3H), 1.30-1.90 ppm (m, 9H), 0.85-0.95 ppm (m, 3H).

5.5.4 [6]-Gingerdiol

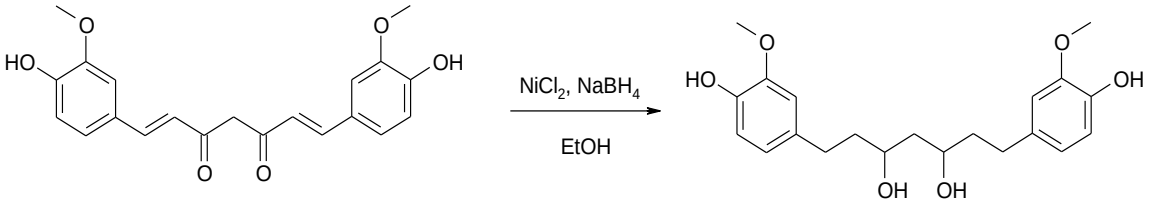


Dissolve 0.32 g of $NaBH_4$ in 20 ml of ethanol, cool down with an ice bath. Add the solution of 2.5 g of [6]-gingerol in 10 mL of ethanol (dropwise) to the mixture and keep the temperature around 0°C. After addition, remove the cooling bath and stir at room temperature for 4 h. Cool down again to 0°C, add 10 mL of 1 N HCl (dropwise) to decompose any excess of $NaBH_4$. Transfer the mixture to a separatory funnel, add 100 ml of brine, and extract with 200 ml of EtOAc. Wash the organic layer with brine twice and dry with $MgSO_4$.

The crude was purified by flash chromatography (silica gel, EtOAc/Hexanes), 1.2 g of product is obtained and the yield is 47.7%. 400-MHz ^1H NMR ($CDCl_3$) δ: 6.77-6.84 ppm (m, 1H), 6.62-6.74 ppm (m, 2H), 5.56 ppm (br. s, 1H), 3.83-4.34 ppm

(m, 2H), 3.87 ppm (4s, 3H), 2.57-2.79 ppm (m, 2H), 1.37-1.92 ppm (m, 6H), 1.15-1.37 ppm (m, 6H), 0.83-0.91 (m, 3H). 400-MHz ^{1}H NMR ($CDCl_3$) of the starting material, [6]-gingerol: δ 6.81 ppm (d, 1H, J = 8.00 Hz), 6.67 ppm (s, 1H, J = 1.92 Hz), 6.64 ppm (d, 1H, J = 8.04 Hz, of d, J = 1.92 Hz), 5.73 ppm (br. s, 1H), 3.86 ppm (br. s, 1H), 3.85 ppm (3s, 3H), 3.04 ppm (br. s, 1H), 2.80-2.86 ppm (m, 2H), 2.70-2.76 ppm (m, 2H), 2.45-2.59 ppm (m, 2H), 1.23-1.50 ppm (m, 8H), 0.88 (t, 3H, J = 6.88 Hz).

5.5.5. Octahydrocurcumin



Dissolve 5 g of curcumin in 250 ml of ethanol and 56 mL of water. Start stirring, add 3.23 g of nickel chloride ($NiCl_2$) to the mixture and cool down to 0 °C. Under N_2 atmosphere, add 4.11 g of sodium borohydride to the mixture within 1 h. After stirring at 0 °C for 5 h, neutralize the reaction mixture with 0.4 N HCl to pH 4. Transfer the mixture to a separatory funnel and extract with 200 ml of dichloromethane. Wash the organic layer with brine twice and dry with $MgSO_4$. After filtration and concentration, 6.3 g of crude product is obtained.

After purifying 1 g using the *BioTage* (an automated preparative HPLC), 0.41 g was purified to 80% purity. 400-MHz ^{1}H NMR (CD_3OD) δ: 6.77 ppm (d, 2H, J = 1.72 Hz), 6.73 ppm (d, 2H, J = 8.00 Hz), 6.64 ppm (d, 2H, J = 8.04 Hz, of d, J = 1.76 Hz),

3.83 ppm (m, 2H), 3.80 ppm (s, 6H), 2.54-2.73 ppm (m, 4H), 1.69-1.78 ppm (m, 4H), 1.56-1.60 ppm (m, 2H).

5.6. Sensory Evaluation of Lemon Oils

5.6.1. Sensory Evaluation of Lemon Oils after Thermal Acceleration

After being stored in the thermally accelerated storage chamber at 90.7°F for four weeks, each lemon oil was tasted at 100ppm in a high-acid tasting solution with a pH of 2.56. The panel consisted of five experts in the field: Dennis Kujawski [Director of Flavor Creations], Richard Dandrea [Senior Flavorist], Hedy Kulka [Senior Flavorist], Anusha Sampath [Flavorist Trainee] and Sharon Tortola [Junior Flavorist]. Each flavorist was given a list of sensory attributes (specifically pertaining to citrus) and was asked to rate each attribute on the scale from "0" to "9", with "0" representing no flavor impact perceived and "9" representing the greatest flavor impact imaginable.

5.6.2. Sensory Evaluation of Lemon Beverages after Thermal Acceleration

Each lemon oil (of *set one*) was added to a high-acid tasting solution with a pH of 2.56 at 100 ppm and tasted after being stored in the thermally accelerated storage chamber at 90.7°F for two weeks. The panel consisted of seven experts in the field: Dennis Kujawski [Director of Flavor Creations], Richard Dandrea [Senior Flavorist], Hedy Kulka [Senior Flavorist], Anusha Sampath [Flavorist Trainee],

Sharon Tortola [Junior Flavorist] and myself, Kathryn Bardsley [Junior Flavorist]. Each flavorist was given a list of sensory attributes (specifically pertaining to citrus) and was asked to rate each attribute on the scale from "0" to "9", with "0" representing no flavor impact perceived and "9" representing the greatest flavor impact imaginable.

5.7. Statistical Analysis of Lemon Oils and Peroxide Values

5.7.1. Statistical Analysis of Peroxide Values

The data analyses were performed using the *One-Way ANOVA* using *JMP's Fit Model*, with the peroxide value being the dependent and the samples of oils as the independent variables. The *Post-Hoc* was the Student's T-Tests with a significance of a 95% Confident Level (p=0.05). The same was used for the paired comparisons of the lemon oils: set one versus set two.

5.7.2. Statistical Analysis of Tasting Results

The data analyses were performed using the *Two-Way ANOVA* using *JMP's Fit Model*, with the ratings being the dependent and the samples of oils and panelists as the independent variables (using JMP's default effects). The *Post-Hoc* was the Student's T-Tests with a significance of a 95% Confident Level (p=0.05).

6. RESULTS AND DISCUSSION

6.1. General Background Information

6.1.1. Growing Regions of Ginger Samples

All (nine) ginger powders are *Zingiber officinale* Roscoe and are discussed below with their growing regions and harvesting methods.

"Synthite Rajakumari" is grown in the state of Kerala, India, at an altitude around 3000 to 5000 feet. Once the ginger is harvested, it is dried on rocks and the skin is peeled manually. "Synthite Irutti" is obtained from low altitude areas of Kerala (north side) and is sold mostly with the skin on. "Synthite Shimoga" is obtained from a high altitude area in the neighboring state Karnataka in north east India. All of the before mentioned rhizomes were harvested between December of 2009 and February of 2010; after which, the ginger is washed and dried.

"Synthite Nigerian" is grown in Kafanja, northern Nigeria, in the Kaduna area and was harvested in February of 2010. The ginger grown here is typically sold by Nigeria for extraction, grinding and/ or trade and is purchased in its dried, whole form after it has been cleaned for stones and filth and subsequently ground. The ground ginger does not contain carriers or additives and is processed in Synthite's factory.

"Whole Herb Nigerian" ginger is collected from various regions by local farmers from the following five states of the Federation namely, Kaduna, Nasarawa, Benue, Niger and Gombe, with Kaduna being the major producer. The ginger was harvested between January and April in 2009. "Whole Herb Indian" ginger is grown

in Kerala and was also harvested between January and April in 2010. The rhizomes are air dried, peeled and ground to a fine powder without the use of carriers.

"Buderim Australian" ginger is grown in Queensland which is about 100-130 miles from Brisbane and was harvested in 2008. "Buderim Fijian" ginger is grown within 40 miles from the plant, located in Suva, Fiji and was harvested in 2009. The ginger is harvested around late June/ early July, dried using drum-drying and subsequently ground, without the use of carriers.

Lastly, "PharmaChem Chinese" ginger is grown in China, however, no additional information was provided.

6.1.2. Moisture Content of Ginger Powders

Sample	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Average Moisture Content	11.63%	11.22%	10.14%	10.58%	8.88%	10.89%	8.97%	9.42%	11.45%

Table 1. Average moisture content of ginger powders

The moisture content of Whole Herb Nigerian was the lowest value out of the set, followed by the two Buderim samples, Australian and Fijian. Of the nine ginger powders, the three with the lowest moisture contents are also the three that were harvested the earliest (between 2008 and 2009); therefore, dehydration is likely occurring during storage. In addition, from the processing information received from the vendors, Buderim was the only company that reported the use drum-drying, which may also contribute to the low moisture content values.

6.1.3. Sensory Descriptions of Ginger Samples

6.1.3.1. Aroma of Dried Ginger Powders

Synthite Rajakumari: dark brown notes, cooked ginger, spicy, Indian food, fresh, warm.

Synthite Irutti: chocolate, sweet, ginger snaps, herbal, turmeric-like, cocoa, slightly fruity, citrus, cheap chocolate.

Synthite Nigerian: sharp citrus, fresh, pungency of fresh ginger, lemon-lime, candied, fruity, soft profile.

Synthite Shimoga: earthy, mushroom, moldy, cheesy, pungent with citrus undertones, muddy, less fresh, dirty, raw mushrooms, unwashed lettuce, fishy, ammonia.

Whole Herb Nigerian: spicy, savory, sage, chicken rub, citrus, very unique, powdery, lacks freshness, common ginger, slight vitamin note.

Whole Herb Indian: freeze dried, refrigerator smell, slight candied note, cooked ginger, lacks freshness, herbal, slightly dirty, slight mushroom.

Buderim Australian: woody, sandalwood, aroma reminiscent of an old church, balanced, not fresh, sugary, *Nestea* iced tea mix, choking.

Buderim Fijian: typical ginger powder, hay, not fresh, cosmetic, lemon juice, soft, lacks pungency.

PharmaChem Chinese: fresh, clean, slight citrus, lemon-lime, ginger, hay, slightly animalic, pungent, earthy, turmeric-like, cinnamon, nasal warming, cooked ginger, methional, chocolate undertones, butyraldehyde, valeraldehyde.

6.1.3.2. Blotter Aroma of 10% Dilutions in Acetone

Synthite Rajakumari: sulfurous, citrus, marigold, asafetida, liver, brussels sprout, alliaceous, green, vegetable, processed meat.

Synthite Irutti: household cleaner, limonene, perfume, nutty, walnut hulls, earthy, cocoa bean, more volatile than the others, drying, malt, heavy, fixative.

Synthite Nigerian: herbaceous, furfural, sweet, gingerbread cookies, maltol, citronellal, waxy at end, warm, cinnamic, baker's cinnamon, resinous, pungency of mustard powder (thiocyanate-like), eugenol, anise.

Synthite Shimoga: leafy, wet leaves, green fatty aldehydes, slight mint, coumarin, cardboard, pencil shavings, cedrol, grease/ fat, slight citrus.

Whole Herb Nigerian: animalic, less fresh smelling than Synthite's Nigerian ginger, phenolic, cured meat, not reminiscent of ginger, spice house, earthy, old dried herbs, woody, cardamom, masculine, citral, very lemony.

Whole Herb Indian: ginger powder, slight terpeney, black pepper-like, maple, sweet potato, methional, brown, candied, powdery.

Buderim Australian: fermented, animalic (like a gutted deer and digested grass), musky, hay, grainy, dried fruit (*Craisins*), slight civet, cat urine.

Buderim Fijian: sweaty, brown, caramel, musty, hay, broom, furfural, coumarin, fatty acids, slight cinnamon notes, slight guaiacol on dry down.

PharmaChem Chinese: geraniol, citrus, fresh ginger, phenolic (Band-aid), pungent, slight eugenol, typical ginger character, end of profile is similar to beginning of Synthite Nigerian with warm, spicy notes.

6.1.3.3. Tasting Comments of 0.1% Solutions in Water

Synthite Rajakumari: lime, heat builds significantly, fecal, manure, skatol, animalic, processed meats.

Synthite Irutti: capsicum heat, woody.

Synthite Nigerian: warm, brown ginger, warming, tingle.

Synthite Shimoga: citral, floral, a lot of heat, stemmy, woody.

Whole Herb Nigerian: floral, much less heat than Synthite's Nigerian, bland, delayed tingle.

Whole Herb Indian: perfume, soapy, weak, slightly warming.

Buderim Australian: very floral, old ginger (not fresh tasting), moderate heat, coumarinic, hay.

Buderim Fijian: brown, perfume, sand, a lot of heat, bitter, tingle.

PharmaChem Chinese: typical ginger flavor, fast onset of heat/ tingle on tip of tongue, flat.

After compiling the aroma, flavor and taste descriptors of each, one may conclude that discernable differences amongst sensory attributes may suggest differences in the chemical composition between the ginger samples. In addition, one may be able to taste which ginger has the highest concentration of gingerols and shogaols based on a greater sensation of heat (ie. Synthite Nigerian) and lastly, is the favorable pairing of ginger with citrus, as a few (ie. Whole Herb Nigerian and Synthite Shimoga), have inherent lemon-like characteristics.

6.1.4. Extraction of Ginger Powders

6.1.4.1. Acetone Extracts Using Orbital Shaker

Orbital Shaker Acetone Extract	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Yield; Percent Yield	1.51g; 5.03%	1.43g; 4.77%	1.81g; 6.03%	1.64g; 5.47%	2.04g; 6.8%	1.59g; 5.3%	1.31g; 4.37%	1.5g; 5%	1.61g; 5.37%

Table 2. Yields of acetone extracts using orbital shaker

6.1.4.2. Hexane Extracts Using Speed Extractor

Speed Extractor Hexane Extract	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Starting Material	30.68g	35.07g	28.46g	28.39g	30.21g	25.76g	44g	32.31g	30.74g
Yield; Percent Yield	1.09g; 3.55%	1.02g; 3.58%	1.08g; 3.79%	1.62g; 5.71%	1.19g; 3.94%	1.03g; 4%	0.84g; 1.91%	0.94g; 2.91%	1.12g; 3.64%

Table 3. Yields of hexane extracts using speed extractor

6.1.5. Analytical Work: GC and HPLC

The profiling of each ginger sample has been completed by both gas chromatography (GC) and high-performance liquid chromatography (HPLC). The GC results for the acetone extracts are listed alphabetically and by retention time, along with their chromatograms, and can be found in the appendix (Appendices 1-

11). In addition, the HPLC chromatograms of both hexane and acetone extracts can also be found in the appendix (Appendices 12-20 and 21-29, respectively). Both profiles of the GCs and HPLCs are comparable to one another and the profiles of the ginger samples themselves are similar, except for the two Buderim ginger powders. Both Buderim samples were deficient in α-zingiberene, which is probably a result of the processing conditions concerning drum-drying.

6.2. Ginger Selection

6.2.1. Determination of Gingerol and Shogaol Content

To compare the gingerol and shogaol content of each, the peak areas (from the HPLC chromatograms) of [6]-, [8]- and [10]-gingerols and [6]-, [8]- and [10]-shogaols were added together. To simplify the data, the summations are ranked in the table below from "1" to "9", with "1" representing the largest summation of gingerols and shogaols and "9" representing the smallest (Table 4). (Please see Appendix 30 for the peak areas of each.)

It was determined that Buderim Fijian ginger, followed by Synthite Nigerian, had the highest amount of gingerols and shogaols out of the hexane extracts. For the acetone extracts, both Nigerian samples (Synthite and Whole Herb Company, respectively) had the largest summation.

Ranking of Gingerols & Shogaols	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Hexane Peak Areas	8	9	2	4	3	6	7	1	5
Acetone Peak Areas	6	5	1	8	2	7	9	4	3

Table 4. Ranking of gingerol and shogaol summation

6.2.2. Antioxidant Activity Studies

6.2.2.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a method used to determine the efficiency of antioxidants in scavenging free radicals. DPPH is a stable free radical, violet in color and has an absorbance of 517nm. Below depicts the scheme for the assay and demonstrates two methods of termination: the hydrogen donating ability of an antioxidant (AH) and the association of two radicals, which results in the reduction of the DPPH radical. Once the DPPH radical is reduced, the absorbance will decrease and turn yellow in color (Brand-Williams *et al*., 1995).

$$DPPH\bullet + AH \rightarrow DPPH\text{-}H + A\bullet$$

$$DPPH\bullet + A\bullet \rightarrow DPPH\text{-}A$$

Scheme 2. The reduction of DPPH

From the results listed in Table 5, the acetone extracts were able to scavenge DPPH to a greater extent than the hexane extracts. The acetone extract of Synthite

Nigerian was able to scavenge nearly 88% of the DPPH radical while the hexane extract was able to scavenge 55.4%. The highest scavenging activity for the hexane extracts was 60% by Whole Herb Nigerian.

DPPH Scavenging Activity (%)	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Hexane Extracts	21.9	25.4	55.4	45.8	60	50.3	40.1	55.4	41.5
Acetone Extracts	57.8	55.7	87.9	39	32.9	33	56.4	50.9	59

Table 5. DPPH scavenging activity of ginger extracts

6.2.2.2. Oxygen Radical Absorbance Capacity (ORAC)

The ORAC assay is based on fluorescence which is quenched by peroxyl radicals. To determine the effectiveness of an antioxidant, the inhibition time to prolong the quenching of the fluorescent probe is measured.

The concentrations of the ginger extracts were selected by employing a range of levels from 0.0001% to 5% to determine the best fit of the decay curve. The ORAC fluorescence curves of each ginger extract at 0.01% can be found in the appendix (Appendices 31 and 32) while Table 6 depicts the values of antioxidant activity.

ORAC Values	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma-Chem Chinese
Hexane Extracts	2.20	0.44	1.19	1.90	2.46	1.94	0.10	2.38	1.73
Acetone Extracts	2.89	3.55	5.39	2.34	4.50	1.31	0.79	3.87	3.72

Table 6. ORAC values for hexane and acetone extracts at 0.01%

6.2.2.3. Summary of Hexane Extracts versus Acetone Extracts

Hexane Extracts	Peak Area Values	Peak Area Ranking	DPPH Values	DPPH Ranking	ORAC Values	ORAC Ranking
Synthite Rajakumari	38399	8	21.9	9	2.20	3
Synthite Irutti	34409	9	25.4	8	0.44	8
Synthite Nigerian	64578	2	55.4	2/3	1.19	7
Synthite Shimoga	57718	4	45.8	5	1.90	5
Whole Herb Nigerian	61472	3	60	1	2.46	1
Whole Herb Indian	52244	6	50.3	4	1.94	4
Buderim Australian	42023	7	40.1	7	0.10	9
Buderim Fijian	68996	1	55.4	2/3	2.38	2
Pharma-Chem	56474	5	41.5	6	1.73	6

Table 7. Compilation of hexane extracts: values and rankings

Acetone Extracts	Peak Area Values	Peak Area Ranking	DPPH Values	DPPH Ranking	ORAC Values	ORAC Ranking
Synthite Rajakumari	83808	6	57.8	3	2.89	6
Synthite Irutti	88144	5	55.7	5	3.55	5
Synthite Nigerian	99649	1	87.9	1	5.39	1
Synthite Shimoga	79563	8	39	7	2.34	7
Whole Herb Nigerian	93751	2	32.9	9	4.50	2
Whole Herb Indian	80246	7	33	8	1.31	8
Buderim Australian	69144	9	56.4	4	0.79	9
Buderim Fijian	91112	4	50.9	6	3.87	3
Pharma-Chem	93515	3	59	2	3.72	4

Table 8. Compilation of acetone extracts: values and rankings

Tables 7 and 8 depict all before-mentioned values, as well as, the assigned rankings for discussion. In Table 7, the ranking of hexane peak areas parallel with the ranking of DPPH but are less aligned with the ORAC results. In Table 8, the ranking of acetone peak areas parallel with the ranking of ORAC but are less aligned with DPPH. One can see how effective gingerols and shogaols are in both assays, as the acetone extracts have a greater concentration and exude greater DPPH scavenging, along with higher ORAC values. However, the discrepancies between the ORAC and DPPH rankings for each solvent, demonstrates that other

constituents, with differing polarities, are being extracted along with the gingerols and shogaols which may enhance the antioxidant efficacy of ginger.

After observing the peak area summations along with the results of the antioxidant assays, the acetone extract of Synthite Nigerian ginger was selected to be studied more in depth.

6.3. Fractionation and ORAC Monitoring

6.3.1. Ginger Extract Fractionation via SepBox®

The SepBox® system is a unique approach in fractionating plant material. It is a two dimensional HPLC which combines preparative-scale HPLC with solid phase extraction (SPE). The SepBox® is fully automated and can fractionate up to five grams of plant material within 24 hours covering the entire spectrum of polarities and recovering sufficient material for structure elucidation. The method employed was developed by IFF for the fractionation of botanical extracts, which requires a reversed-phase column; therefore, the selected ginger extract had to be first washed with hexanes before extracting with acetone. The hexane washed acetone extract of Synthite Nigerian yielded 316 fractions with high purity.

6.3.2. ORAC Assay of Each Fraction

Each of the 316 fractions was tested for its ORAC value and the top 10 fractions having the highest ORAC values were listed in Table 9.

Top 10	Fraction Number	ORAC Value
1	126	11.55
2	111	11.48
3	313	11.48
4	142	11.4
5	167	11.39
6	310	11.36
7	308	11.33
8	127	11.32
9	312	11.29
10	164	11.25

Table 9. Synthite Nigerian SepBox® fractions with the highest ORAC values

Also included, are the ORAC values of known antioxidants found in ginger (Table 10). The concentrations reported refer to the concentrations added to the assay; however, after all other reagents are added to each well, the concentration of the extract is diluted to 12.5% of the initial concentration.

Table 10 demonstrates how antioxidant activity is strongly dependent on concentration; all activities of solutions greater than 0.1% and below 0.01% diminish greatly while the range between 0.01 and 0.1% proves to be optimum. The diminished activity of the 1% dilutions also supports the theory that when a concentration is too high, an antioxidant will become a "pro-oxidant." Last to mention is the diminished activity of the antioxidants when increasing their chain length which directly supports the contradiction made earlier: increasing the chain length does not necessarily increase the antioxidant activity.

Initial Concentration Added to Well	Final Concentration in Well	[6]-Gingerol	[6]-Shogaol	[10]-Gingerol	[10]-Shogaol
1%	0.125%	8.85	5.14	5.94	-0.72
0.1%	0.0125%	11.08	11.18	8.39	8.30
0.01%	0.00125%	11.16	11.21	6.40	5.81
0.001%	0.000125%	3.61	1.11	-1.09	-1.39

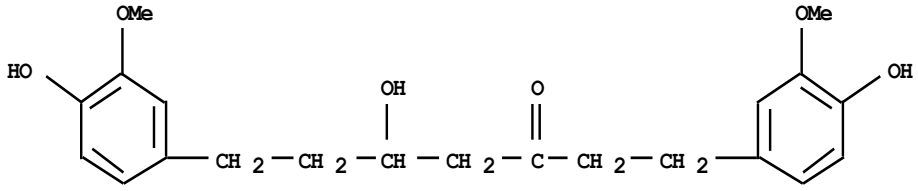
Table 10. ORAC values for known ginger antioxidants

6.3.3. Identification of Antioxidants

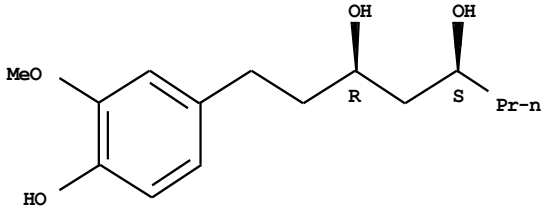
6.3.3.1 Proposed Active Compounds

Select ginger fractions were chosen for identification based on their ORAC values and the quantity available. Using the developed LC/MS/UV method, MS/MS, HRMS and referencing the literature (Jiang *et al*., 2007), several compounds were proposed as the major components of the chosen fractions. The following fractions were among the top 10 but could not be used for identification due to sample size: F313, 310, 308 and 312. The most abundant fraction was F166 which was used to discern the selected concentration of 5000 ppm for analysis and was later found to be [6]-gingerol. Fractions 126, 111, 142, 167, 110, 165 and 108 were identified (Figures 6a-i; Table 11) and the corresponding LC and exact mass chromatograms can be found in the appendix (Appendices 33-35).

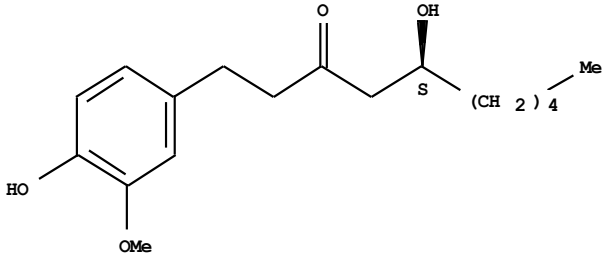
a. F126/ F111: Hexahydrocurcumin (5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)- 3-heptanone)



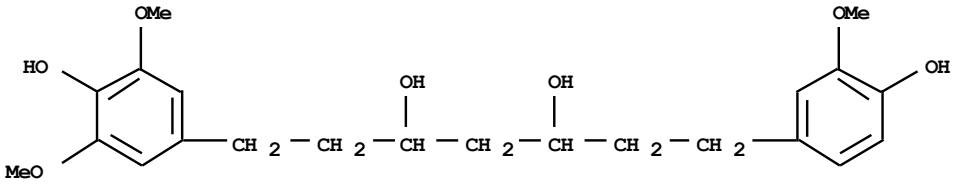
b. F142: [4]-Gingerdiol ((3R,5S)- 1-(4-hydroxy-3-methoxyphenyl)-3,5-octanediol)



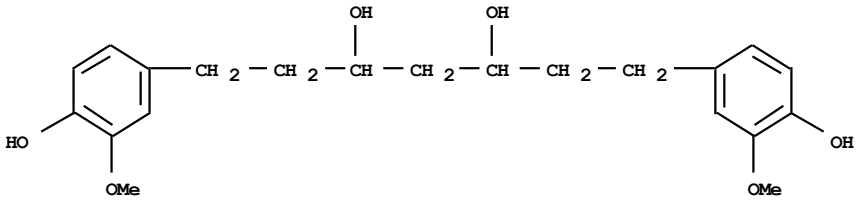
c. F167: [6]-Gingerol ((5S)- 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone)



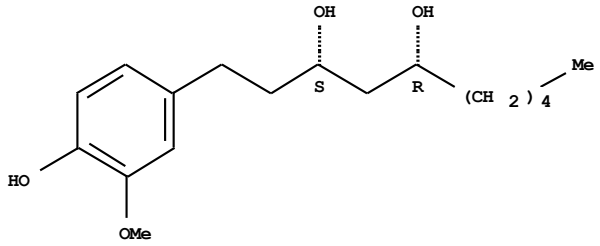
d. F110-1: 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3,5-heptanediol



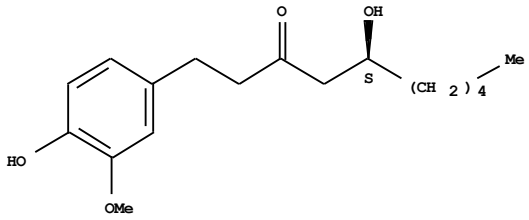
e. F110-2: Octahydrocurcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5 heptanediol)



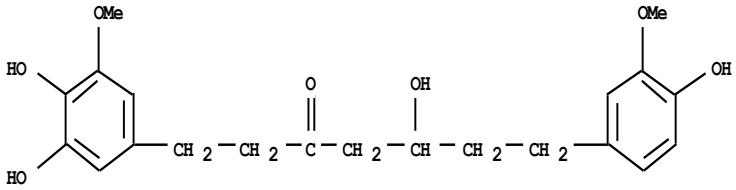
f. F165-1: [6]-Gingerdiol ((3S,5R)- 1-(4-hydroxy-3-methoxyphenyl)-3,5-decanediol)



g. F165-2: [6]-Gingerol ((5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone)



h. F108-1: 1-(3,4-dihydroxy-5-methoxyphenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone)



i. F108-2: 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-heptanone

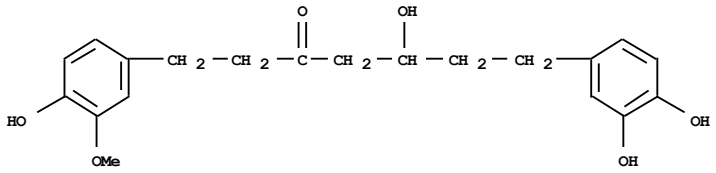


Figure 6. Structures (a-i) of the identified compounds

Fraction Number	Retention Time (min)	(M+H)+	APCI (ms/ms)+	Molecular Formula	Identification
126	18.87	405.1911	207	C22H28O7	F126-1*
	19.56	375.1806	177	C21H26O6	Hexahydrocurcumin
111	18.87	405.1911	207	C22H28O7	F111-1* = F126-1*
142	16.91	297.206	279, 261	C17H28O4	F142-1*
	17.98	341.2324	323, 305, 287	C19H32O5	F142-2*
	19.2	269.1748	251, 233, 177, 163	C15H24O4	[4]-Gingerdiol
167	25.72	491.2285	431, 371, 177	C26H34O9	F167-1*
	26.14	295.1902	277, 177	C17H26O4	[6]-Gingerol
110	18.33	407.2058	371, 389, 193, 167	C22H30O7	F110-1*
	19.17	377.1955	341, 359, 163, 217	C21H28O6	Octahydrocurcumin
165	26.04	297.2058	279, 261, 163, 137	C17H28O4	[6]-Gingerdiol
	27.33	295.1904	277, 177	C17H26O4	[6]-Gingerol
108	18.01	391.1749	373, 193	C21H26O7	F108-1*
	18.26	361.1645	343, 179	C20H24O6	F108-2*

*Names were too long to include in table but can be found next to their corresponding structures.

Table 11. Active compounds identified from Synthite Nigerian ginger fractions

6.3.3.2 Confirmed Active Compounds

The racemic structures of the proposed compounds: hexahydrocurcumin, [6]-gingerol, [4]-gingerdiol, octahydrocurcumin and [6]-gingerdiol from ginger fractions F126/ 111, F167, F142, F110 and F165, respectively, were confirmed using commercial standards (hexahydrocurcumin and [6]-gingerol) and an IFF synthetic standards ([4]-gingerdiol, octahydrocurcumin and [6]-gingerdiol).

For F126/ 111 and F167, based on LC/MS/MS, HRMS and information reported in literature, were proposed to be hexahydrocurcumin and [6]-gingerol,

respectively. In terms of LC retention time and MS2 spectra, the peak of F126/111 and that of F167, matched very well with the commercial standards and the proposed structures were confirmed. The LC/MS/MS profiles and ^{1}H-NMR spectra can be found in the appendix (Appendices 36- 39).

The same has been done for F142, F110 and F165; based on LC/MS/MS, HRMS and information obtained from literature, the synthetic isomers of the standards matched very well (Appendices 40-45).

6.4. Use of Antioxidants in Lemon Oil

6.4.1 Determination of Antioxidant Concentrations via ORAC

Based on the ORAC values for the known antioxidants of ginger (Table 10), a range of concentrations to be added to the wells were selected for each antioxidant: [4]-gingerol, [4]-shogaol and [4]-gingerdiol (0.001% to 1%), [6]-gingerol and [6]-gingerdiol (0.006% to 1%), *S*-[6]-gingerol (0.008 to 0.6%), tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin (0.0001 to 1%) and mixed tocopherols (0.00006 to 0.1%). (Please refer to Appendix 46 for the levels employed in the assay with their corresponding ORAC values.) Although tetrahydrocurcumin, [4]-gingerol and [4]-shogaol were not identified as being one of the top 10 antioxidants, because they are analogs of those identified, they too have been included in the study (^{1}H-NMR Appendices 47-49).

6.4.2 Antioxidants in Lemon Oil

A single-fold Argentinian lemon oil, cold-pressed by Brown extraction, was chosen for the stability study. The following table (Table 12) depicts the selected levels of antioxidants added to the lemon oil. The sets were created in triplicate and stored in amber glass bottles at 90.7°F. Two control sets were also created: one placed in the thermally accelerated storage chamber with the other samples and the other, kept in the refrigerator. All samples were removed after four weeks.

Antioxidant:	Concentration in Lemon Oil (Set 1):	Concentration in Lemon Oil (Set 2):
4-gingerol	12.5 ppm	100 ppm
4-gingerdiol	12.5 ppm	100 ppm
4-shogaol	12.5 ppm	100 ppm
6-gingerol	125 ppm	1000 ppm
S-6-gingerol	125 ppm	
6-gingerdiol	75 ppm	600 ppm
6-shogaol	125 ppm	
8-gingerol	125 ppm	
8-shogaol	125 ppm	
10-gingerol	125 ppm	
10-shogaol	125 ppm	
Tetrahydrocurcumin	50 ppm	400 ppm
Hexahydrocurcumin	125 ppm	
Octahydrocurcumin	25 ppm	200 ppm
Mixed Tocopherols	250 ppm	1000 ppm

Table 12. Levels of antioxidants added to Argentinian lemon oil

6.4.3 Peroxide Study of the Lemon Oils with and without Ginger-related Antioxidants

The starting oil has an average peroxide value of 12.6 meq (milliequivalents of peroxides per 1000 g of oil), in which, a peroxide value up to 20 meq is deemed acceptable by taste. Table 13 depicts the mean peroxide value for each sample.

	Set 1:		Set 2:	
Antioxidant:	**Concentration of Antioxidant in Lemon Oil:**	**Peroxide Value of Lemon Oil:**	**Concentration of Antioxidant in Lemon Oil:**	**Peroxide Value of Lemon Oil:**
Blank Control, Refrigerated		15.86 meq		
Blank Control, Thermally Accelerated		19.1 meq		
4-gingerol	12.5 ppm	21.84 meq	100 ppm	20.2 meq
4-gingerdiol	12.5 ppm	22.87 meq	100 ppm	24.59 meq
4-shogaol	12.5 ppm	21.72 meq	100 ppm	22.66 meq
6-gingerol	125 ppm	23.04 meq	1000 ppm	38.78 meq
S-6-gingerol	125 ppm	34.79 meq		
6-gingerdiol	75 ppm	21.4 meq	600 ppm	35.48 meq
6-shogaol	125 ppm	25.65 meq		
8-gingerol	125 ppm	25.32 meq		
8-shogaol	125 ppm	21.03 meq		
10-gingerol	125 ppm	24.18 meq		
10-shogaol	125 ppm	26.69 meq		
Tetrahydrocurcumin	50 ppm	18.68 meq	400 ppm	30.8 meq
Hexahydrocurcumin	125 ppm	21.49 meq		
Octahydrocurcumin	25 ppm	29.49 meq	200 ppm	28.53 meq
Mixed Tocopherols	250 ppm	15.26 meq	1000 ppm	25.96 meq

Table 13. Mean peroxide values of Argentinian lemon oils

For the first set, the samples containing mixed tocopherols and tetrahydrocurcumin were the most effective at prohibiting peroxide formation. However, the increasing peroxide values for the samples containing antioxidants versus the thermally accelerated blank control, indicates that the concentration of antioxidants employed are too high.

For the second set, all levels employed were too high; however, compared to the other samples in this set, 4-gingerol, 4-shogaol and 4-gingerdiol were most

effective. When optimizing the concentrations in the ORAC assay (Appendix 46), 4-gingerol, 4-shogaol and 4-gingerdiol did not vary greatly which is evident in the lemon oil study as well; employing nearly ten times the antioxidant yielded nearly the same peroxide value (concentrations in set one versus set two).

In contrast yet supporting the statement made earlier, the results of tocopherols (250 ppm versus 1000 ppm) proves that using a concentration of an antioxidant well above optimum efficacy results in the generation of a pro-oxidant.

Another interesting observation is the linear relationship between peroxide value and ethanol content. The samples *S*-6-gingerol (from set 1), 6-gingerol, 6-gingerdiol and tetrahydrocurcumin (from set 2) have elevated levels of ethanol and significantly higher peroxide values (Appendices 50 and 51). This indicates that there may be trace metals in the ethanol which may have a catalytic effect triggering auto-oxidation or ethanol itself, may be a pro-oxidant.

Through statistical data analysis, it was determined that there weren't significant differences between the control oils (refrigerated blank and thermally accelerated blank) after heat treatment, however, there were significant differences between the thermally accelerated control oil and a few samples containing antioxidants. Tables 14 through 16 were included to display the degree of significance between all peroxide values of the oils; the bar charts corresponding to these tables can be found in the appendix (Appendices 52-54). Please note, that samples sharing a common letter in the column labeled "Multiple Comparisons" denotes that there is no significant difference between them.

Antioxidant	N Rows	Mean	Std Errors	Multiple Comparisons
Tocopherols	3	15.26	2.65	i
Blank, Refrigerated		15.86		
Tetrahydrocurcumin	3	18.68	0.98	hi
Blank, Hot Box	2	19.10	2.58	ghi
8-shogaol	3	21.03	0.71	fgh
6-gingerdiol	3	21.40	1.77	efgh
Hexahydrocurcumin	3	21.49	1.37	efgh
4-shogaol	3	21.72	0.49	defgh
4-gingerol	3	21.84	1.31	defgh
4-gingerdiol	3	22.87	0.56	cdefg
6-gingerol	3	23.04	0.93	cdefg
10-gingerol	3	24.18	0.72	cdef
8-gingerol	3	25.32	1.96	cde
6-shogaol	3	25.65	1.38	bcd
10-shogaol	3	26.69	1.25	bc
Octahydrocurcumin	3	29.49	1.31	b
S-6-gingerol	3	34.79	1.68	a

p < 0.05: significant difference between the two samples at p=0.05 (95% confidence); NS : No significant difference at p>0.05

Table 14. Data analysis of peroxide values from first set of lemon oils

Antioxidant	N Rows	Mean	Std Errors	Multiple Comparisons
4-gingerol	3	20.20	1.62	f
4-shogaol	3	22.66	0.28	ef
4-gingerdiol	3	24.59	1.24	def
Tocopherols	3	25.96	1.00	cde
Octahydrocurcumin	2	28.53	0.11	cd
Tetrahydrocurcumin	3	30.80	1.40	bc
6-gingerdiol	3	35.48	2.14	ab
6-gingerol	3	38.78	3.30	a

p < 0.05: significant difference between the two samples at p=0.05 (95% confidence); NS : No significant difference at p>0.05

Table 15. Data analysis of peroxide values from second set of lemon oils

Antioxidant	N Rows	Mean	Std Errors	Paired Comparisons
Blank, Hot Box	2	19.10	2.58	
Blank, Refrigerated		15.86		
4-gingerdiol (12.5ppm)	3	22.87	0.56	NS (p=0.28)
4-gingerdiol (100ppm)	3	24.59	1.24	
4-gingerol (12.5ppm)	3	21.84	1.31	NS (p=0.47)
4-gingerol (100ppm)	3	20.20	1.62	
4-shogaol (12.5ppm)	3	21.72	0.49	NS (p=0.17)
4-shogaol (100ppm)	3	22.66	0.28	
6-gingerdiol (75ppm)	3	21.40	1.77	p=0.01
6-gingerdiol (0.06%)	3	35.48	2.14	
6-gingerol (125ppm)	3	23.04	0.93	p=0.01
6-gingerol (0.1%)	3	38.78	3.30	
Octahydrocurcumin (25ppm)	3	29.49	1.31	NS (p=0.61)
Octahydrocurcumin (200ppm)	2	28.53	0.11	
Tetrahydrocurcumin (50ppm)	3	18.68	0.98	p=0.00
Tetrahydrocurcumin (400ppm)	3	30.80	1.40	
Tocopherols (250ppm)	3	15.26	2.65	p=0.02
Tocopherols (0.1%)	3	25.96	1.00	

$p < 0.05$: significant difference between the two samples at p=0.05 (95% confidence); NS : No significant difference at $p>0.05$

Table 16. Paired comparison of significance between sets of lemon oils

As stated earlier, 6-gingerdiol, 6-gingerol and tetrahydrocurcumin from the second set, have elevated amounts of ethanol and from the statistical analysis, one can see that this increase has a significantly negative impact.

Regarding 4-gingerdiol, 4-gingerol and 4-shogaol, there is no significant difference which proves that the concentration level for these is less sensitive. Appendix 46 depicts that changing the concentration from 40 ppm to 10,000 ppm does not yield a significant change in ORAC value. Similarly to the "four moieties", the peroxide values of octahydrocurcumin demonstrate no significant difference between 25 ppm and 200 ppm which parallels the ORAC values (Appendix 46) at 200 ppm and 1600 ppm (representing 12.5 % of the concentration employed into

the oil). This is not the case, however, regarding tocopherols as one can see in Table 16; increasing the level beyond its optimum concentration drastically increases the peroxide value.

6.4.4. Sensory Validation of Ginger-related Antioxidants in Lemon Drink

The following attributes of the lemon oils (Table 17) were rated by an expert citrus panel consisting of five to seven flavorists, on a scale from "0" to "9", with "0" representing no flavor impact perceived and "9" representing the greatest flavor impact imaginable.

Descriptors:
Peely/ Lemon Peel
Citral
Candied
Juicy
Oxidized
Cooked
Plastic/ Phenolic
Cherry-like/ Medicinal
Piney
Soapy/ Green
Turpentine

Table 17. Lemon beverage descriptors

The lemon oils with a peroxide value closest to the mean peroxide value of the set were selected for tasting. All oils were dosed into the high-acid tasting medium at 100 ppm.

6.4.4.1. Sensory Validation of Lemon Oils after Thermal Treatment

From the data analysis of the sensory ratings, it was determined that there were no significant differences overall, between the refrigerated lemon oil and the

oils stored in the chamber after four weeks, therefore, the complete list of tasting results has not been reported. However, there were significant differences between the oils regarding the single attribute of *citral* (Figure 7). Interestingly, the most favored tasting solutions were those incorporating tocopherols and tetrahydrocurcumin which were also the two oils with the lowest peroxide values.

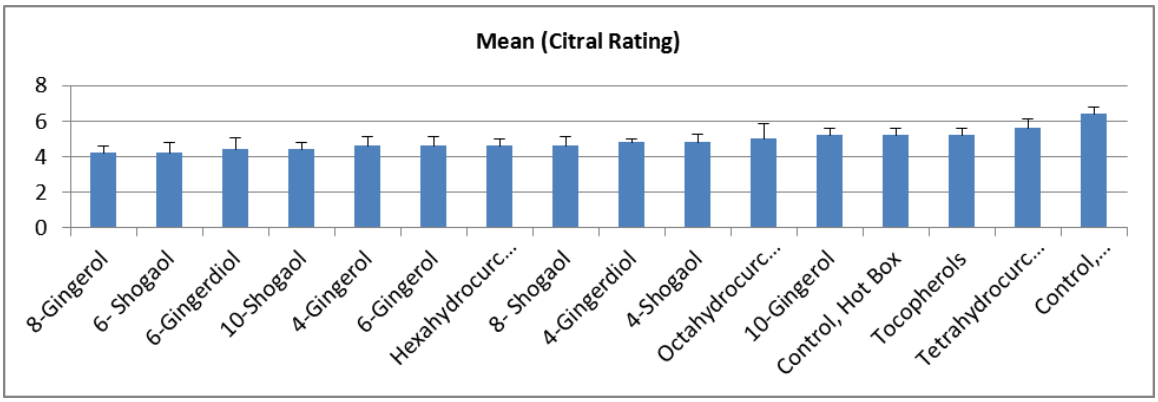


Figure 7. Citral ratings of lemon oils after storage

6.4.4.2. Sensory Validation of Lemon Beverages after Thermal Treatment

A second tasting was conducted by a trained panel consisting of seven flavorists, using the same oils at 100ppm but after the high-acid tasting solutions containing the oils were stored in the chamber for an additional two weeks.

Representing the three control beverages, the following terms were used: *hot box, refrigerated,* and *fresh*, which refer to the lemon beverage stored in the thermally accelerated chamber for two weeks, the lemon beverage stored in the refrigerator for two weeks and the lemon beverage made on the morning of the tasting, respectively; none of which, contained antioxidants.

For the attribute, *peely*, the fresh and refrigerated samples were significantly more peely than the others but not significantly different from each other. In addition, none of the samples containing antioxidants were significantly more peely than the hot box sample (Table 18).

Attribute	Sample	Mean	Std Err	Significance
Peely	Fresh Blank	6.43	0.48	a
Peely	Refrigerated Blank	6.00	0.76	a
Peely	4-Gingerol 12.75ppm	4.71	0.47	b
Peely	6-Shogaol 123.5ppm	4.29	0.78	bc
Peely	Hexahydrocurcumin 125ppm	4.29	0.64	bc
Peely	4-Gingerdiol 12.5ppm	4.14	0.55	bc
Peely	4-Shogaol 13.5ppm	3.86	0.83	bcd
Peely	10-Shogaol 125ppm	3.71	0.99	bcd
Peely	6-Gingerdiol 79.7ppm	3.71	0.78	bcd
Peely	Mixed Tocopherols 497ppm	3.71	0.61	bcd
Peely	Octahydrocurcumin 26.7ppm	3.71	0.71	bcd
Peely	Hot Box Blank	3.57	0.61	bcd
Peely	Tetrahydrocurcumin 51.25ppm	3.43	0.75	cd
Peely	6-Gingerol 126ppm	3.29	0.68	cd
Peely	8-Shogaol 123.7ppm	3.29	0.52	cd
Peely	10-Gingerol 122.4ppm	3.14	0.77	cd
Peely	8-Gingerol 121ppm	2.71	0.64	d

Table 18. Statistical analysis of lemon beverage tastings for the attribute, peely

For *citral*, only hexahydrocurcumin and octahydrocurcumin had a significantly greater citral impact than the hot box sample. All other samples containing antioxidants had significantly less citral flavor than the refrigerated sample and were not significantly different from the hot box sample. Lastly, the refrigerated and fresh samples were significantly different from one another which depicts how unstable citral is in an acidic environment (Table 19).

Attribute	Sample	Mean	Std Err	Significance
Citral	Fresh Blank	6.86	0.34	a
Citral	Refrigerated Blank	5.43	0.69	b
Citral	Hexahydrocurcumin 125ppm	4.29	0.52	c
Citral	Octahydrocurcumin 26.7ppm	4.00	0.58	cd
Citral	10-Shogaol 125ppm	3.86	0.74	cde
Citral	4-Shogaol 13.5ppm	3.86	0.86	cde
Citral	8-Shogaol 123.7ppm	3.71	0.61	cdef
Citral	4-Gingerol 12.75ppm	3.57	0.48	cdef
Citral	6-Gingerdiol 79.7ppm	3.57	0.87	cdef
Citral	6-Shogaol 123.5ppm	3.57	0.57	cdef
Citral	Tetrahydrocurcumin 51.25ppm	3.43	0.53	cdef
Citral	10-Gingerol 122.4ppm	3.29	0.61	cdef
Citral	Mixed Tocopherols 497ppm	3.29	0.64	cdef
Citral	4-Gingerdiol 12.5ppm	3.14	0.67	def
Citral	8-Gingerol 121ppm	2.86	0.51	ef
Citral	Hot Box Blank	2.86	0.59	ef
Citral	6-Gingerol 126ppm	2.71	0.84	f

Table 19. Statistical analysis of lemon beverage tastings for the attribute, citral

For the attribute, *candied*, 4-gingerol, 6-gingerdiol and 8-shogaol were as candied as the refrigerated sample and significantly different from the hot box. The remaining samples were not significantly different from the hot box sample but 6-shogaol, 10-shogaol, 4-shogaol, tocopherols, 10-gingerol, 6-gingerol and hexahydrocurcumin were not significantly different from the refrigerated sample either. All samples, including the refrigerated sample, were significantly less candied compared to the fresh lemon beverage (Table 20).

Attribute	Sample	Mean	Std Err	Significance
Candied	Fresh Blank	6.00	0.58	a
Candied	Refrigerated Blank	4.86	0.74	b
Candied	4-Gingerol 12.75ppm	4.71	0.52	bc
Candied	6-Gingerdiol 79.7ppm	4.29	0.78	bcd
Candied	8-Shogaol 123.7ppm	4.29	0.64	bcd
Candied	6-Shogaol 123.5ppm	4.14	0.55	bcdef
Candied	10-Shogaol 125ppm	4.00	0.62	bcdef
Candied	4-Shogaol 13.5ppm	4.00	0.82	bcdef
Candied	Mixed Tocopherols 497ppm	4.00	0.58	bcdef
Candied	10-Gingerol 122.4ppm	3.86	0.59	bcdef
Candied	6-Gingerol 126ppm	3.86	0.80	bcdef
Candied	Hexahydrocurcumin 125ppm	3.86	0.51	bcdef
Candied	4-Gingerdiol 12.5ppm	3.71	0.87	cdef
Candied	Octahydrocurcumin 26.7ppm	3.57	0.65	def
Candied	Tetrahydrocurcumin 51.25ppm	3.29	0.52	def
Candied	Hot Box Blank	3.14	0.74	ef
Candied	8-Gingerol 121ppm	3.00	0.72	f

Table 20. Statistical analysis of lemon beverage tastings for the attribute, candied

As depicted in Table 21, 4-gingerol and hexahydrocurcumin were juicier than the hot box sample and not significantly different than the refrigerated sample. In addition, 4-gingerol was the only sample besides the refrigerated sample that was as juicy as the fresh. Out of the remaining samples, only 6-shogaol was as juicy as both the hot box and refrigerated samples, while all other samples were significantly different from the refrigerated sample and not significantly different from the hot box sample.

Attribute	Sample	Mean	Std Err	Significance
Juicy	Fresh Blank	4.71	0.52	a
Juicy	Refrigerated Blank	4.43	0.57	ab
Juicy	4-Gingerol 12.75ppm	3.57	0.75	abc
Juicy	Hexahydrocurcumin 125ppm	3.43	0.72	bcd
Juicy	6-Shogaol 123.5ppm	3.29	0.64	bcde
Juicy	10-Shogaol 125ppm	3.14	0.40	cde
Juicy	4-Gingerdiol 12.5ppm	2.86	0.74	cde
Juicy	4-Shogaol 13.5ppm	2.86	0.70	cde
Juicy	Mixed Tocopherols 497ppm	2.86	0.77	cde
Juicy	10-Gingerol 122.4ppm	2.71	0.52	cde
Juicy	6-Gingerdiol 79.7ppm	2.71	0.71	cde
Juicy	8-Shogaol 123.7ppm	2.71	0.47	cde
Juicy	Octahydrocurcumin 26.7ppm	2.71	0.81	cde
Juicy	Tetrahydrocurcumin 51.25ppm	2.71	0.36	cde
Juicy	6-Gingerol 126ppm	2.57	0.65	cde
Juicy	8-Gingerol 121ppm	2.29	0.68	de
Juicy	Hot Box Blank	2.14	0.40	e

Table 21. Statistical analysis of lemon beverage tastings for the attribute, juicy

For the attribute, *oxidized*, none of the samples containing antioxidants were significantly different from the hot box sample; however, 4-gingerdiol, 10-shogaol, 6-gingerdiol, 4-shogaol, octahydrocurcumin, 6-gingerol, 6-shogaol, 4-gingerol and hexahydrocurcumin were not significantly more oxidized than the refrigerated sample. All beverages, including the refrigerated lemon beverage, were significantly more oxidized than the fresh, which depicts how sensitive a panelist are towards perceiving oxidation (Table 22).

Attribute	Sample	Mean	Std Err	Significance
Oxidized	8-Gingerol 121ppm	5.14	0.74	a
Oxidized	10-Gingerol 122.4ppm	4.43	0.95	ab
Oxidized	Tetrahydrocurcumin 51.25ppm	4.29	0.71	abc
Oxidized	Hot Box Blank	4.14	1.12	abc
Oxidized	8-Shogaol 123.7ppm	4.00	0.62	abc
Oxidized	Mixed Tocopherols 497ppm	3.71	0.81	abc
Oxidized	4-Gingerdiol 12.5ppm	3.57	0.78	bcd
Oxidized	10-Shogaol 125ppm	3.43	0.75	bcd
Oxidized	6-Gingerdiol 79.7ppm	3.29	1.02	bcd
Oxidized	4-Shogaol 13.5ppm	3.14	0.70	bcd
Oxidized	Octahydrocurcumin 26.7ppm	3.14	0.70	bcd
Oxidized	6-Gingerol 126ppm	3.00	0.93	bcd
Oxidized	6-Shogaol 123.5ppm	3.00	0.65	bcd
Oxidized	4-Gingerol 12.75ppm	2.86	0.86	cd
Oxidized	Hexahydrocurcumin 125ppm	2.86	0.86	cd
Oxidized	Refrigerated Blank	2.14	0.77	d
Oxidized	Fresh Blank	0.14	0.14	e

Table 22. Statistical analysis of lemon beverage tastings for the attribute, oxidized

In Table 23, 6-shogaol, hexahydrocurcumin and 10-shogaol were significantly less cooked tasting than the hot box sample but not significantly worse than the refrigerated sample. 4-Gingerdiol was the only sample that was not significantly different from either, the hot box or the refrigerated sample.

Attribute	Sample	Mean	Std Err	Significance
Cooked	Hot Box Blank	4.86	0.70	a
Cooked	Mixed Tocopherols 497ppm	4.43	0.65	ab
Cooked	Octahydrocurcumin 26.7ppm	4.43	0.53	ab
Cooked	10-Gingerol 122.4ppm	4.29	0.78	ab
Cooked	8-Gingerol 121ppm	4.29	0.71	ab
Cooked	4-Shogaol 13.5ppm	4.14	0.59	ab
Cooked	6-Gingerdiol 79.7ppm	4.14	0.77	ab
Cooked	6-Gingerol 126ppm	4.14	0.46	ab
Cooked	8-Shogaol 123.7ppm	4.14	0.46	ab
Cooked	4-Gingerol 12.75ppm	4.00	0.93	abc
Cooked	Tetrahydrocurcumin 51.25ppm	4.00	0.79	abc
Cooked	4-Gingerdiol 12.5ppm	3.86	0.77	abcd
Cooked	6-Shogaol 123.5ppm	3.57	0.78	bcd
Cooked	Hexahydrocurcumin 125ppm	3.00	0.62	cd
Cooked	Refrigerated Blank	3.00	1.02	cd
Cooked	10-Shogaol 125ppm	2.86	0.67	d
Cooked	Fresh Blank	1.14	0.70	e

Table 23. Statistical analysis of lemon beverage tastings for the attribute, cooked

For the attribute, *plastic or phenolic*, all samples, excluding the fresh sample, were statistically similar to the hot box sample; however, octahydrocurcumin, 10-shogaol and the refrigerated, were the only samples that were not significantly different from the fresh sample (Table 24).

Attribute	Sample	Mean	Std Err	Significance
Plastic/ Phenolic	10-Gingerol 122.4ppm	2.29	1.08	a
Plastic/ Phenolic	4-Shogaol 13.5ppm	2.29	0.94	a
Plastic/ Phenolic	6-Shogaol 123.5ppm	2.14	0.96	a
Plastic/ Phenolic	6-Gingerdiol 79.7ppm	2.00	0.87	a
Plastic/ Phenolic	6-Gingerol 126ppm	2.00	0.95	a
Plastic/ Phenolic	Tetrahydrocurcumin 51.25ppm	2.00	0.82	a
Plastic/ Phenolic	4-Gingerol 12.75ppm	1.86	0.59	a
Plastic/ Phenolic	8-Gingerol 121ppm	1.86	0.86	a
Plastic/ Phenolic	Mixed Tocopherols 497ppm	1.86	0.80	a
Plastic/ Phenolic	4-Gingerdiol 12.5ppm	1.71	0.71	a
Plastic/ Phenolic	8-Shogaol 123.7ppm	1.71	0.71	a
Plastic/ Phenolic	Hot Box Blank	1.71	0.94	a
Plastic/ Phenolic	Hexahydrocurcumin 125ppm	1.57	0.81	a
Plastic/ Phenolic	Octahydrocurcumin 26.7ppm	1.43	0.81	ab
Plastic/ Phenolic	10-Shogaol 125ppm	1.29	0.64	ab
Plastic/ Phenolic	Refrigerated Blank	1.14	0.86	ab
Plastic/ Phenolic	Fresh Blank	0.14	0.14	b

Table 24. Statistical analysis of lemon beverage tastings for the attribute, plastic/phenolic

For *cherry or medicinal*, none of the samples were significantly different from the hot box except for the refrigerated and fresh sample. 8-gingerol, 6-gingerdiol, 10-gingerol and 6-gingerol, however, had significantly more cherry and medicinal off-notes than the refrigerated sample. In addition, hexahydrocurcumin, 6-shogaol, 10-shogaol, 4-gingerdiol and 4-shogaol were the only samples to not have significant differences compared to the fresh lemon beverage (Table 25).

Attribute	Sample	Mean	Std Err	Significance
Cherry/ Medicinal	8-Gingerol 121ppm	2.00	0.76	a
Cherry/ Medicinal	6-Gingerdiol 79.7ppm	2.00	0.85	a
Cherry/ Medicinal	10-Gingerol 122.4ppm	2.00	0.90	a
Cherry/ Medicinal	Hot Box Blank	1.86	0.70	a
Cherry/ Medicinal	6-Gingerol 126ppm	1.86	0.96	a
Cherry/ Medicinal	Octahydrocurcumin 26.7ppm	1.57	0.75	ab
Cherry/ Medicinal	Mixed Tocopherols 497ppm	1.57	0.65	ab
Cherry/ Medicinal	Tetrahydrocurcumin 51.25ppm	1.43	0.57	ab
Cherry/ Medicinal	8-Shogaol 123.7ppm	1.43	0.61	ab
Cherry/ Medicinal	4-Gingerol 12.75ppm	1.43	0.69	ab
Cherry/ Medicinal	Hexahydrocurcumin 125ppm	1.29	0.64	abc
Cherry/ Medicinal	6-Shogaol 123.5ppm	1.29	0.81	abc
Cherry/ Medicinal	10-Shogaol 125ppm	1.29	0.57	abc
Cherry/ Medicinal	4-Gingerdiol 12.5ppm	1.14	0.59	abc
Cherry/ Medicinal	4-Shogaol 13.5ppm	1.00	0.49	abc
Cherry/ Medicinal	Refrigerated Blank	0.57	0.43	bc
Cherry/ Medicinal	Fresh Blank	0.29	0.29	c

Table 25. Statistical analysis of lemon beverage tastings for the attribute, cherry/medicinal

Other than octahydrocurcumin, 6-gingerol, 6-shogaol, 8-shogaol and tocopherols, all samples containing antioxidants had significantly less piney off-flavor than the hot box sample. In addition, except for 4-shogaol and 4-gingerol, the remaining samples were statistically equivalent to the fresh lemon beverage (Table 26).

Attribute	Sample	Mean	Std Err	Significance
Piney	Hot Box Blank	3.86	0.83	a
Piney	Octahydrocurcumin 26.7ppm	3.29	0.81	ab
Piney	6-Gingerol 126ppm	2.86	0.59	abc
Piney	6-Shogaol 123.5ppm	2.86	0.26	abc
Piney	8-Shogaol 123.7ppm	2.86	0.70	abc
Piney	Mixed Tocopherols 497ppm	2.86	0.51	abc
Piney	4-Shogaol 13.5ppm	2.71	0.57	bc
Piney	4-Gingerol 12.75ppm	2.57	0.61	bc
Piney	10-Gingerol 122.4ppm	2.43	0.72	bcd
Piney	6-Gingerdiol 79.7ppm	2.43	0.57	bcd
Piney	Refrigerated Blank	2.43	0.61	bcd
Piney	Tetrahydrocurcumin 51.25ppm	2.43	0.37	bcd
Piney	10-Shogaol 125ppm	2.29	0.71	bcd
Piney	Hexahydrocurcumin 125ppm	2.29	0.71	bcd
Piney	4-Gingerdiol 12.5ppm	2.14	0.63	cd
Piney	8-Gingerol 121ppm	2.00	0.58	cd
Piney	Fresh Blank	1.43	0.30	d

Table 26. Statistical analysis of lemon beverage tastings for the attribute, piney

For the attribute, *soapy or green*, only 10-shogaol, hexahydrocurcumin and 6-shogaol were significantly different from the hot box sample, however, none (except for the hot box sample) were significantly different from neither the fresh nor the refrigerated sample (Table 27).

Attribute	Sample	Mean	Std Err	Significance
Soapy/ Green	Hot Box Blank	2.57	0.84	a
Soapy/ Green	10-Gingerol 122.4ppm	2.00	0.85	ab
Soapy/ Green	4-Gingerol 12.75ppm	2.00	0.62	ab
Soapy/ Green	6-Gingerdiol 79.7ppm	2.00	0.82	ab
Soapy/ Green	6-Gingerol 126ppm	2.00	0.82	ab
Soapy/ Green	8-Shogaol 123.7ppm	1.86	0.86	ab
Soapy/ Green	Mixed Tocopherols 497ppm	1.86	0.59	ab
Soapy/ Green	Refrigerated Blank	1.86	0.80	ab
Soapy/ Green	Tetrahydrocurcumin 51.25ppm	1.86	0.59	ab
Soapy/ Green	4-Shogaol 13.5ppm	1.71	0.57	ab
Soapy/ Green	8-Gingerol 121ppm	1.71	0.84	ab
Soapy/ Green	Octahydrocurcumin 26.7ppm	1.71	0.64	ab
Soapy/ Green	4-Gingerdiol 12.5ppm	1.57	0.72	ab
Soapy/ Green	10-Shogaol 125ppm	1.29	0.42	b
Soapy/ Green	Hexahydrocurcumin 125ppm	1.29	0.36	b
Soapy/ Green	Fresh Blank	1.14	0.46	b
Soapy/ Green	6-Shogaol 123.5ppm	1.00	0.31	b

Table 27. Statistical analysis of lemon beverage tastings for the attribute, soapy/green

For the attribute, *turpentine*, octahydrocurcumin, tetrahydrocurcumin, 8-shogaol, 10-shogaol and hexahydrocurcumin were all significantly less turpentine-like in flavor compared to the hot box. In addition, 8-shogaol, the refrigerated sample, 10-shogaol and hexahydrocurcumin were also not significantly different from the fresh sample which demonstrates favorable stabilization of the lemon oils by these antioxidants (Table 28).

Attribute	Sample	Mean	Std Err	Significance
Turpentine	Hot Box Blank	3.14	0.99	a
Turpentine	Mixed Tocopherols 497ppm	3.14	0.86	a
Turpentine	4-Gingerol 12.75ppm	3.00	0.76	ab
Turpentine	6-Gingerol 126ppm	2.57	0.84	abc
Turpentine	6-Gingerdiol 79.7ppm	2.43	0.65	abc
Turpentine	6-Shogaol 123.5ppm	2.43	0.53	abc
Turpentine	4-Gingerdiol 12.5ppm	2.29	0.75	abc
Turpentine	10-Gingerol 122.4ppm	2.14	0.40	abc
Turpentine	8-Gingerol 121ppm	2.14	0.59	abc
Turpentine	4-Shogaol 13.5ppm	2.00	0.58	abcd
Turpentine	Octahydrocurcumin 26.7ppm	1.71	0.57	bcde
Turpentine	Tetrahydrocurcumin 51.25ppm	1.71	0.61	bcde
Turpentine	8-Shogaol 123.7ppm	1.57	0.53	cdef
Turpentine	Refrigerated Blank	1.43	0.81	cdef
Turpentine	10-Shogaol 125ppm	0.71	0.29	def
Turpentine	Hexahydrocurcumin 125ppm	0.57	0.20	ef
Turpentine	Fresh Blank	0.29	0.18	f

Table 28. Statistical analysis of lemon beverage tastings for the attribute, turpentine

Overall, tocopherols and tetrahydrocurcumin had the lowest peroxide values out of the set but did not lend significant flavor stability to the lemon beverage after being stored in the thermal chamber. The most significant positive differences, however, were observed in the samples containing hexahydrocurcumin and 4-gingerol, which also parallels the peroxide study by having two of the lowest peroxide values out of the set. 10-shogaol and 6-shogaol, were second to hexahydrocurcumin and 4-gingerol, but had two of the highest peroxide values. The spider chart below (Figure 8) depicts the overall flavor perception of the six before-mentioned samples containing antioxidants compared to the three controls and clearly supports the inferences made above.

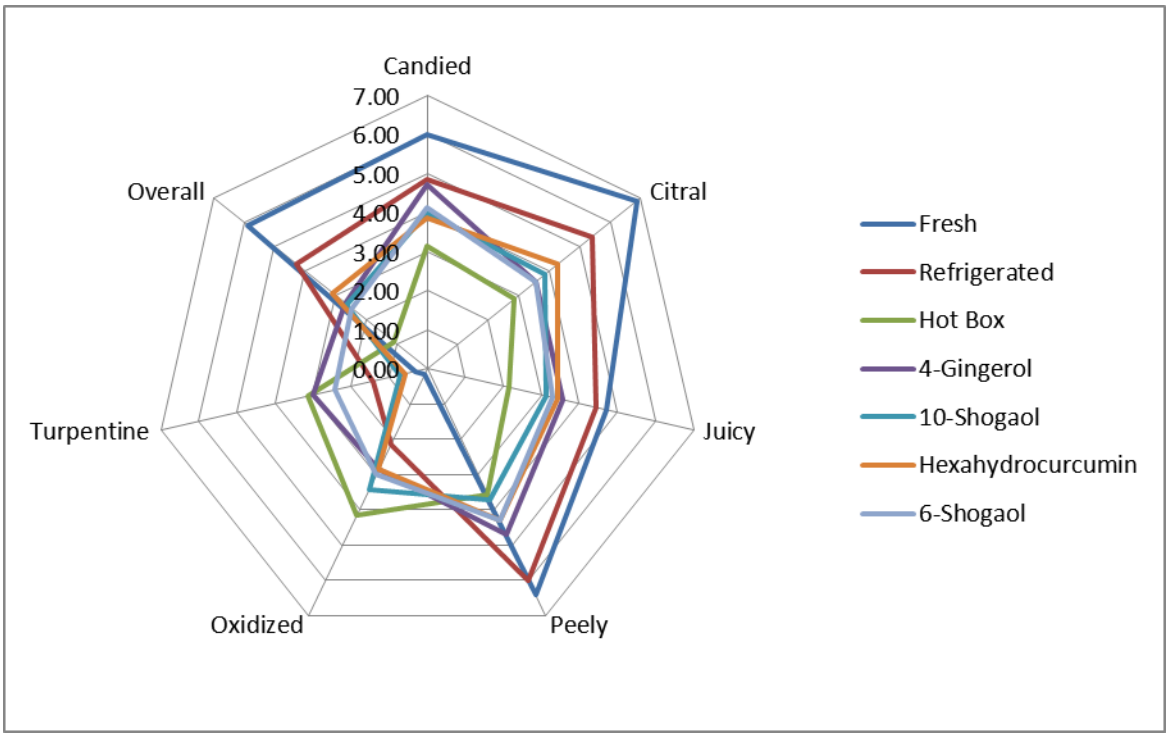


Figure 8: Flavor attributes of most preferred antioxidants in lemon beverages

6.4.5. GC Analysis of Markers Representing Limonene Oxidation and Citral Degradation

The following markers (Table 29) were used to identify which sample of lemon oil was subject to the greatest amount of degradation (Schieberle and Grosch, 1989; Djordjevic *et al.,* 2007; Yang *et al.,* 2011). However, the following markers were not identified in the analysis: cis-*p*-mentha-2,8-dien-1-hydroperoxide, trans-*p*-mentha-2,8-dien-1-hydroperoxide, *p*-mentha-1,8-dien-4-hydroperoxide, trans-*p*-mentha-[1(7),8]-dien-2-hydroperoxide, *p*-mentha-1,8-dien-3-hydroperoxide, trans-*p*-mentha-6,8-dien-2-hydroperoxide, cis-*p*-mentha-6,8-dien-2-hydroperoxide, δ-2-carene, eucarvone, myrtenal, trans-carveol, perillaldehyde, cis-*p*-mentha-[1(7),8]-dien-2-hydroperoxide. In addition, all the following had peak areas of zero: butanoic acid, 2-heptanone, 1-octen-3-ol, p-cresol, α,*p*-dimethylstyrene, *p*-

menthadien-8-ol, thymol, perillyl alcohol, *p*-cymeme-8-ol and *p*-methyl acetophenone.

Literature Degradation Products:
α,*p*-Dimethylstyrene
p-Cymene
p-Methyl acetophenone
p-Cresol
α-Terpineol
2-Heptanone
1-Octen-3-ol
δ-2-Carene
Butanoic acid
1,2-Epoxy-*p*-menth-8-ene
Myrtenal
trans-Carveol
Carvone
Perillyl Alcohol
Perillaldehyde
Limonene Oxide
cis-*p*-Mentha-2,8-dien-1-hydroperoxide
trans-*p*-Mentha-2,8-dien-1-hydroperoxide
p-Mentha-1,8-dien-4-hydroperoxide
trans-*p*-Mentha-[1(7),8]-dien-2-hydroperoxide
p-Mentha-1,8-dien-3-hydroperoxide
trans-*p*-Mentha-6,8-dien-2-hydroperoxide
cis-*p*-Mentha-6,8-dien-2-hydroperoxide
cis-*p*-Mentha-[1(7),8]-dien-2-hydroperoxide

Table 29. Degradation markers for GC/MS analysis of lemon oils

The identified peaks of the tasted lemon oils can be found in Table 30 and the peak areas of all oils, including the GC of the starting oil at t=0, can be found in the appendix (Appendix 55 and 56).

Lemon Oil Sample	*p*-Cymene	Limonene	Limonene oxide	α-Terpineol	Geranial	Neral	Citral	Carvone
Control at t=0	0.19	66.34	0	0.02	2.02	1.17	3.19	0
Control Fridge D	0.28	66.52	0.01	0.02	1.96	1.13	3.09	0.03
Control Hot Box B	0.81	66.43	0.06	0.02	1.94	1.11	3.05	0.03
4-Gingerol C	0.57	66.19	0.04	0.02	1.88	1.1	2.98	0.02
4-Gingerdiol B	0.55	66.17	0.04	0.02	1.9	1.11	3.01	0.02
4-Shogaol C	0.68	66.22	0.05	0.02	1.86	1.09	2.95	0.03
6-Gingerol B	0.53	66.15	0.04	0.02	1.92	1.12	3.04	0.02
6-Gingerdiol A	0.7	66.23	0.06	0.02	1.97	1.14	3.11	0.03
6-Shogaol A	0.73	66.13	0.06	0.02	1.96	1.13	3.09	0.03
8-Gingerol B	0.73	66.19	0.06	0.02	1.89	1.1	2.99	0.02
8-Shogaol A	0.65	66.17	0.06	0.02	1.87	1.09	2.96	0.03
10-Gingerol C	0.76	66.18	0.06	0.02	1.87	1.09	2.96	0.03
10-Shogaol B	0.63	66.22	0.04	0.02	1.76	1.03	2.79	0.02
Tetrahydro-curcumin B	0.73	66.34	0.06	0.02	1.86	1.08	2.94	0.03
Hexahydro-curcumin B	0.76	66.53	0.06	0.02	1.79	1.04	2.83	0.03
Octahydro-curcumin A	0.64	65.75	0.06	0.02	1.79	1.05	2.84	0.03
Tocopherols A	0.58	66.46	0.06	0.02	1.92	1.01	2.93	0.03

Table 30. Peak areas of degradation markers of tasted lemon oils

The inconsistent expectation of the decreased amount of limonene in the control oil at t=0 compared to the heat treated samples is because limonene co-elutes with 1,8-cineole. Therefore, the level of limonene should not be used to compare the oils to one another but rather, limonene oxide instead (Table 30). The two oils with the highest citral peak area are 6-gingerdiol and 6-shogaol; however, the two samples with the highest citral ratings from the first and second tastings were tocopherols and tetrahydrocurucmin (Figure 7), and hexahydrocurcumin and octahydrocurcumin (Table 19), respectively. These findings demonstrate the complexity of the lemon oil and how several constituents may contribute to the perception of a single flavor attribute. Therefore, sensory data has a greater

significance in reflecting the flavor perception than the markers of degradation via the GC.

7. CONCLUSION

To conclude, several observations have been made. The growing region of each ginger powder affects the chemical composition, in which, Nigerian ginger was found to be superior to Australian, Chinese, Fijian and Indian ginger. In addition, processing conditions affect the chemical composition of the botanical and these differences are discernable by taste.

The extraction method also impacts the end result. In compiling the gingerol and shogaol peak areas, one can see that the hexane extracts contain less gingerols and shogaols than the acetone extracts and the acetone extracts have greater values in both assays as compared to the hexane extracts. However, if gingerols and shogaols were the only active participants, the ORAC and DPPH results would align. Their differences indicate that there are more or less polar constituents relative to the gingerols and shogaols that are being extracted and are effective in quenching peroxyl radicals and in scavenging DPPH.

It was determined that four weeks in the thermally accelerated storage chamber was not enough time to produce significant changes in oil degradation. However, increased antioxidant concentrations produced significant increases in peroxide levels, which prove how critical employing the correct concentration is for maximum effectiveness. In addition, it was observed that samples containing increased volumes of ethanol also had significantly higher peroxide values. To discern whether it is the ethanol itself or the presence of trace metals within the

ethanol catalyzing auto-oxidation, additional work must be completed which has been added to the 'future work' section of this composition.

Lastly, after tabulating the sensory results of the beverages after two weeks of storage in the thermally accelerated chamber, hexahydrocurcumin had the most positive impact in stabilizing the flavor quality, which also paralleled with its ORAC and peroxide value. In contrast, however, the mixed tocopherols had the lowest peroxide value and was one of the least preferred lemon beverages. With this said, solely relying on data from objective analyses, such as peroxide values, GC markers, HPLC profiles, ORAC values, DPPH scavenging activity, etc. is not dependable because there are gaps behind the understanding of what and how we taste. Therefore, sensory data is the most complete and reliable data to discern the overall impact an ingredient will have on the subjected flavor profile. To conclude, the identified antioxidants found in Synthite Nigerian ginger had a significantly positive influence on the flavor and stability of lemon oil.

8. FUTURE PERSPECTIVES

Referencing a quote from Paul Schulick, "…an isolated element, thereby exclude[s] the value of the whole herb and the synergy or inherent cooperation [the] wholeness represents" leads me to my future work.

I will repeat the lemon oil stability study and store the samples for a longer period of time, while decreasing the levels of the antioxidants. I will also add new samples to test including the acetone extract of Synthite Nigerian, BHT and ethanol (with and without EDTA to chelate trace metals). Once effective levels are established, I will work with combinations of antioxidants to determine if there are synergistic effects and if they are more powerful that those alone.

In addition, I'd welcome the opportunity to follow the referenced work done by Yang *et al.* (2011) by employing, single or combined antioxidants, in an emulsion system and lastly, I plan to continue identifying those ginger fractions with the highest ORAC values.

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10. APPENDIX

Appendix 1: Chemical Composition of Ginger Oleoresins by GC (Alphabetical)

Component:	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Austra-lian	Buderim Fijian	Pharma-Chem Chinese
1,8-cineole	1.51	0.78	1.79	1.28	0.21	0.44	0.18	0.13	0.10
1-methyl-4-(1,5,9-trimethyl-4-decenyl)-benzene	3.19	3.40	4.11	4.32	3.98	3.73	13.20	8.74	4.82
alpha-amorphene	14.37	18.21	19.72	25.22	20.53	18.21	11.06	16.94	20.55
alpha-copaene	2.31	2.09	2.00	3.04	1.68	2.04	0.36	0.81	2.12
alpha-terpineol	1.86	2.00	3.37	2.94	3.35	1.33	1.78	2.82	2.70
alpha-zingiberene	266.05	255.95	160.27	167.40	137.44	226.6	18.90	25.55	198.44
ar-curcumene	83.19	65.42	67.90	137.57	88.41	109.8	75.61	101.12	73.32
aromadendrene	2.39	2.27	2.32	2.94	2.20	2.67	0.89	1.48	2.32
beta-bisabolene	62.52	60.46	46.18	61.62	50.70	64.33	25.32	33.75	55.28
beta-elemene	3.99	3.75	3.27	5.40	2.30	2.58	0.53	1.21	2.51
beta-elemol	3.55	3.66	3.58	4.51	3.14	1.95	3.57	2.96	2.41
beta-eudesmol	5.50	5.84	0.95	6.48	7.02	6.40	10.16	9.14	5.50
beta-sesquiphellandrene	118.75	116.8	87.73	110.8	92.39	124.3	42.26	57.68	101.49
beta-sesquiphellandrol	17.47	16.90	19.61	16.68	22.84	26.21	26.21	19.63	19.10
borneol	5.68	5.66	6.85	7.36	6.70	5.42	3.21	2.82	5.11
citronellol	1.68	1.83	1.58	1.96	1.15	0.44	2.67	2.42	1.93
decanal	1.15	1.57	1.37	2.94	2.10	0.98	0.89	0.94	1.06
gamma-bisabolene	4.97	6.10	6.01	6.38	6.39	4.53	3.57	5.65	4.82
geraniol	0.53	0.52	1.27	1.08	1.15	0.44	5.71	5.78	5.11
geranyl acetate	0.98	0.87	1.05	1.86	0.94	0.80	0.89	0.94	8.30
germacrene d	3.81	3.75	3.27	4.12	3.46	4.35	2.67	3.23	4.24
hexanal	0.71	0.87	1.05	2.06	1.36	0.44	0.89	1.34	0.87
linalool	4.97	4.09	1.69	1.96	1.15	3.38	0.53	0.54	0.96
sesquisabinene hydrate	7.72	6.45	5.06	7.56	6.91	5.15	8.20	6.72	6.27
trans,trans-alpha-farnesene	29.80	34.15	33.74	27.28	25.67	24.34	3.21	3.09	38.20
trans-beta-farnesene	4.97	4.88	3.58	5.40	3.56	3.55	0.71	0.94	3.86
trans-nerolidol	8.78	9.15	6.12	7.65	6.18	8.62	8.20	8.34	5.98
undecan-2-one	2.39	3.31	1.16	1.67	1.05	0.09	0.53	0.54	1.25
unknown compound bp-137 mw-380	7.63	6.88	12.65	11.19	11.52	6.49	37.80	32.00	14.76
unknown compound bp-171 mw-290	14.99	13.68	12.23	5.20	9.43	20.26	11.95	7.40	10.42
unknown sesquiterpene compound bp-109 mw-238	5.68	6.36	7.49	7.36	8.28	8.53	9.27	6.86	6.08

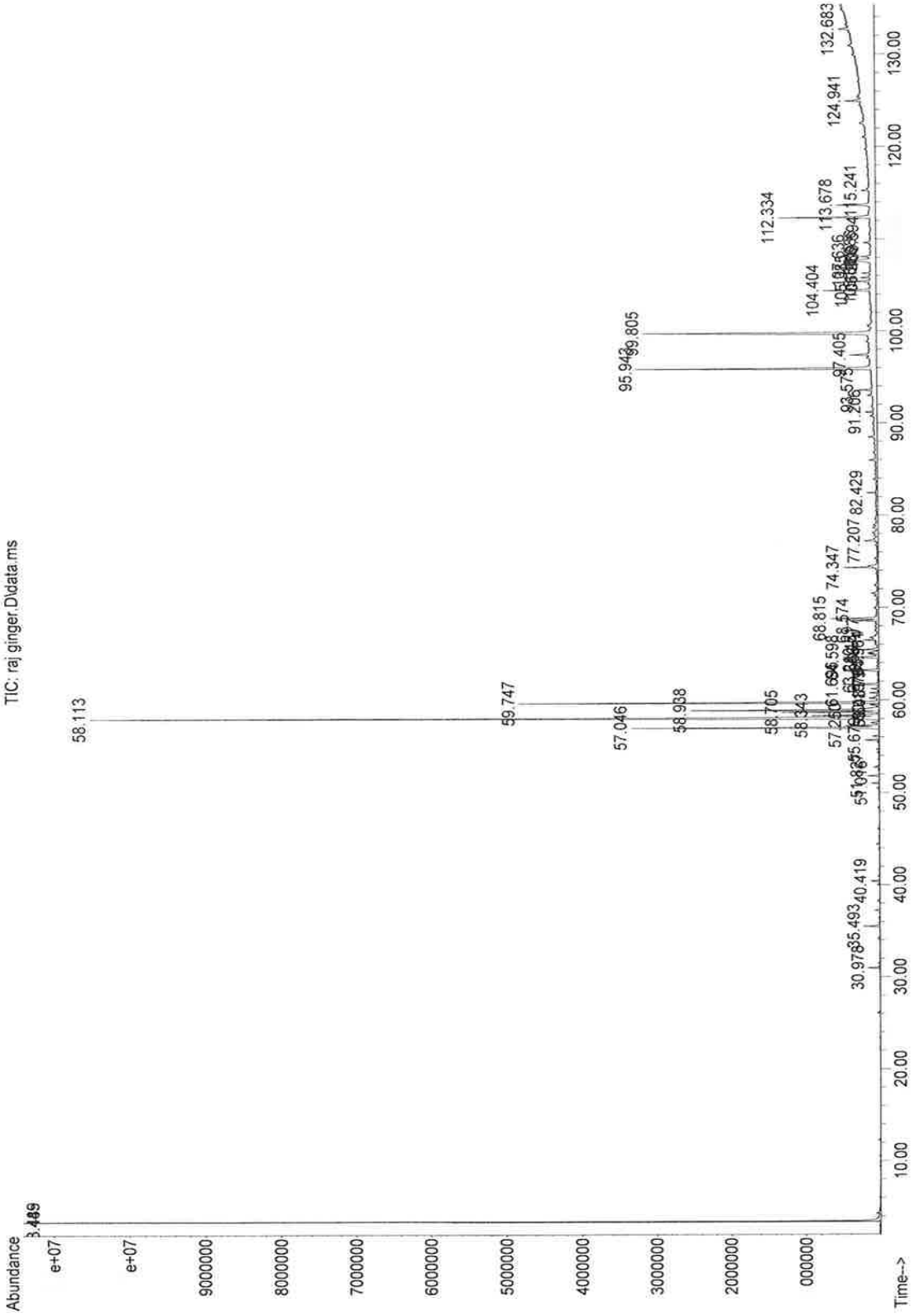
Components continued:	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Austra-lian	Buderim Fijian	Pharma-Chem Chinese
unknown sesquiterpene compound bp-137	10.46	11.33	8.75	11.19	9.43	7.37	9.45	8.07	8.97
unknown sesquiterpene compound bp-69	15.16	15.33	20.24	18.64	18.02	19.37	26.93	19.63	16.30
zingerone	9.93	10.98	8.86	11.28	10.79	7.11	10.88	9.68	9.16
zingiberenol	3.55	4.70	6.33	10.99	7.33	3.73	5.71	6.86	3.86
[10]-gingerdione	14.46	16.29	15.29	10.01	11.21	18.84	16.58	10.76	12.64
[10]-shogaol	25.81	29.71	37.22	30.62	42.11	35.01	77.03	53.25	36.66
[4]-gingerol (t)	1.86	2.18	4.11	1.96	4.71	1.33	2.85	3.36	3.18
[4]-shogaol	1.33	2.87	2.00	1.28	1.89	1.16	4.28	5.11	1.74
[6]-	2.75	2.61	3.80	3.24	3.25	1.78	12.84	5.78	2.80
[6]-gingerdione	8.60	11.59	15.39	6.18	12.47	11.91	13.91	8.47	11.19
[6]-gingerol	109.61	112.03	174.19	118.83	173.69	82.63	123.40	160.68	132.16
[6]-gingerone	4.35	5.49	8.86	9.42	8.90	4.44	15.16	13.04	8.01
[6]-shogaol	65.36	77.01	116.09	79.68	116.49	78.01	279.07	267.98	102.16
[8]-gingerdione	6.47	4.70	9.17	5.40	5.97	7.73	8.02	4.71	6.37
[8]-gingerol (t)	11.26	10.19	14.87	10.21	13.41	10.31	10.34	9.01	12.35
[8]-shogaol	15.96	15.33	25.83	17.86	27.13	20.79	52.60	42.09	22.57
Total Adjusted Parts per Thousand of Named Components	1000.01	1000.0	999.97	1000.0	999.99	999.99	999.98	999.99	1000.00

Appendix 2: Chemical Composition of Ginger Oleoresins by GC (Retention Time)

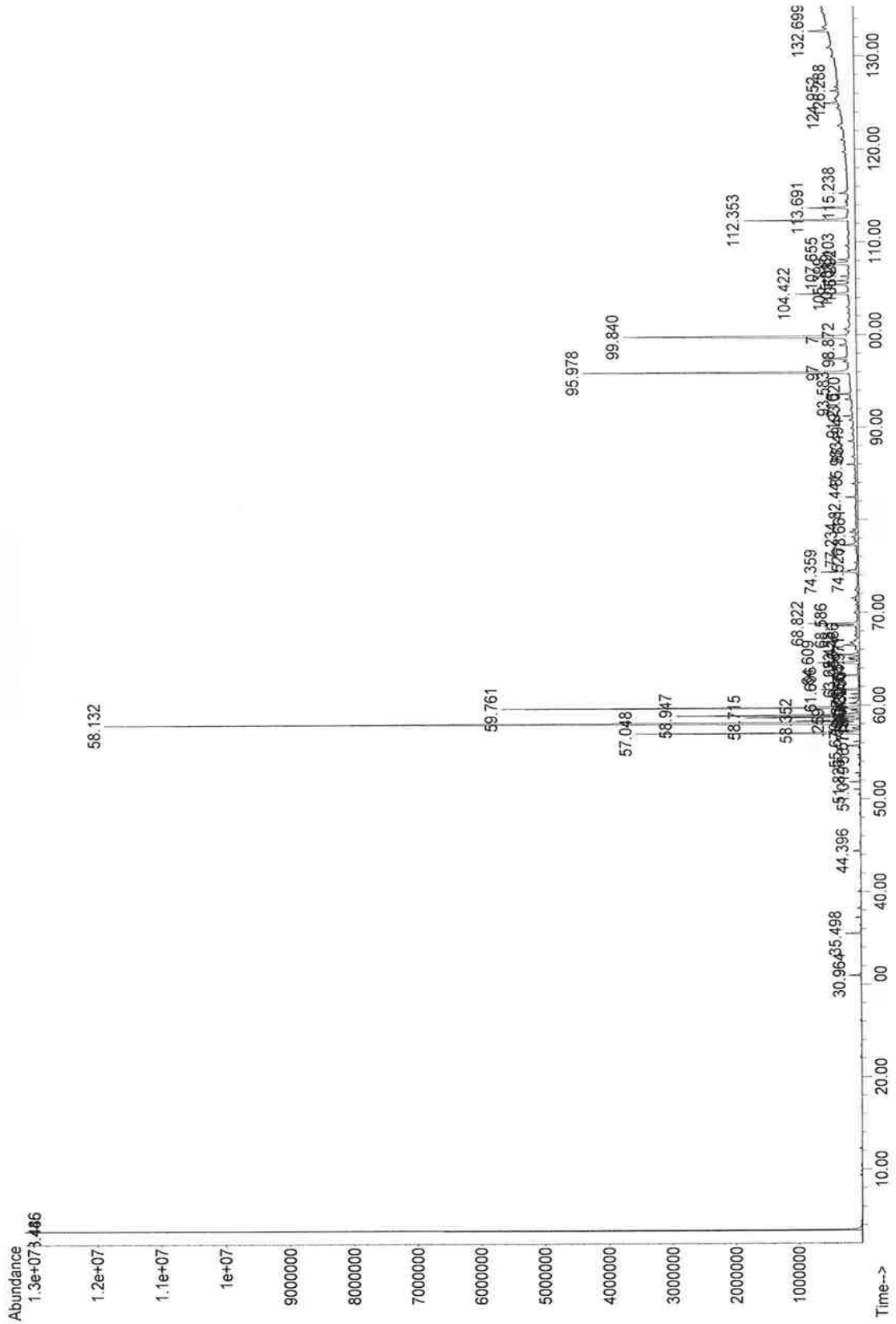
Avg IE	Components	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Austra-lian	Buderim Fijian	PharmaChem Chinese
3.87	hexanal	0.71	0.87	1.05	2.06	1.36	0.44	0.89	1.34	0.87
6.31	1,8-cineole	1.51	0.78	1.79	1.28	0.21	0.44	0.18	0.13	0.10
6.97	linalool	4.97	4.09	1.69	1.96	1.15	3.38	0.53	0.54	0.96
7.59	borneol	5.68	5.66	6.85	7.36	6.70	5.42	3.21	2.82	5.11
7.83	alpha-terpineol	1.86	2.00	3.37	2.94	3.35	1.33	1.78	2.82	2.70
7.98	decanal	1.15	1.57	1.37	2.94	2.10	0.98	0.89	0.94	1.06
8.24	citronellol	1.68	1.83	1.58	1.96	1.15	0.44	2.67	2.42	1.93
8.48	geraniol	0.53	0.52	1.27	1.08	1.15	0.44	5.71	5.78	5.11
8.84	undecan-2-one	2.39	3.31	1.16	1.67	1.05	0.09	0.53	0.54	1.25
9.76	geranyl acetate	0.98	0.87	1.05	1.86	0.94	0.80	0.89	0.94	8.30
9.85	alpha-copaene	2.31	2.09	2.00	3.04	1.68	2.04	0.36	0.81	2.12
9.98	beta-elemene	3.99	3.75	3.27	5.40	2.30	2.58	0.53	1.21	2.51
10.62	trans-beta-farnesene	4.97	4.88	3.58	5.40	3.56	3.55	0.71	0.94	3.86
10.67	aroma-dendrene	2.39	2.27	2.32	2.94	2.20	2.67	0.89	1.48	2.32
10.83	ar-curcumene	83.19	65.42	67.90	137.6	88.41	109.8	75.61	101.1	73.32
10.90	germacrene d	3.81	3.75	3.27	4.12	3.46	4.35	2.67	3.23	4.24
10.99	alpha-zingiberene	266.05	255.95	160.27	167.40	137.44	226.65	18.90	25.55	198.44
11.01	alpha-amorphene	14.37	18.21	19.72	25.22	20.53	18.21	11.06	16.94	20.55
11.11	trans,trans-alpha-farnesene	29.80	34.15	33.74	27.28	25.67	24.34	3.21	3.09	38.20
11.14	beta-bisabolene	62.52	60.46	46.18	61.62	50.70	64.33	25.32	33.75	55.28
11.28	beta-sesquiphellan	118.75	116.82	87.73	110.78	92.39	124.30	42.26	57.68	101.49
11.35	gamma-bisabolene	4.97	6.10	6.01	6.38	6.39	4.53	3.57	5.65	4.82
11.44	beta-elemol	3.55	3.66	3.58	4.51	3.14	1.95	3.57	2.96	2.41
11.61	trans-nerolidol	8.78	9.15	6.12	7.65	6.18	8.62	8.20	8.34	5.98
11.85	sesquisabinene hydrate	7.72	6.45	5.06	7.56	6.91	5.15	8.20	6.72	6.27
12.04	zingiberenol	3.55	4.70	6.33	10.99	7.33	3.73	5.71	6.86	3.86
12.09	zingerone	9.93	10.98	8.86	11.28	10.79	7.11	10.88	9.68	9.16
12.41	beta-eudesmol	5.50	5.84	0.95	6.48	7.02	6.40	10.16	9.14	5.50
12.81	unknown sesquiterpene	10.46	11.33	8.75	11.19	9.43	7.37	9.45	8.07	8.97
12.84	beta-sesquiphellan	17.47	16.90	19.61	16.68	22.84	26.21	26.21	19.63	19.10
13.86	unknown sesquiterpene	15.16	15.33	20.24	18.64	18.02	19.37	26.93	19.63	16.30
14.42	unknown sesquiterpene	5.68	6.36	7.49	7.36	8.28	8.53	9.27	6.86	6.08
15.48	1-methyl-4-(1,5,9-	3.19	3.40	4.11	4.32	3.98	3.73	13.20	8.74	4.82
16.36	[4]-shogaol	1.33	2.87	2.00	1.28	1.89	1.16	4.28	5.11	1.74
16.75	[6]-dehydroshoga	2.75	2.61	3.80	3.24	3.25	1.78	12.84	5.78	2.80
17.22	[4]-gingerol (t)	1.86	2.18	4.11	1.96	4.71	1.33	2.85	3.36	3.18
17.86	[6]-gingerone	4.35	5.49	8.86	9.42	8.90	4.44	15.16	13.04	8.01
18.38	[6]-shogaol	65.36	77.01	116.09	79.68	116.49	78.01	279.07	267.98	102.16

Avg IE Cont'd	Components	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	PharmaChem Chinese
18.66	[6]-gingerdione	8.60	11.59	15.39	6.18	12.47	11.91	13.91	8.47	11.19
19.15	[6]-gingerol	109.61	112.03	174.19	118.83	173.69	82.63	123.40	160.68	132.16
20.09	[8]-shogaol	15.96	15.33	25.83	17.86	27.13	20.79	52.60	42.09	22.57
20.30	unknown compound bp-137 mw-380	7.63	6.88	12.65	11.19	11.52	6.49	37.80	32.00	14.76
20.35	[8]-gingerdione	6.47	4.70	9.17	5.40	5.97	7.73	8.02	4.71	6.37
20.71	unknown compound bp-171 mw-290	14.99	13.68	12.23	5.20	9.43	20.26	11.95	7.40	10.42
20.78	[8]-gingerol (t)	11.26	10.19	14.87	10.21	13.41	10.31	10.34	9.01	12.35
21.58	[10]-shogaol	25.81	29.71	37.22	30.62	42.11	35.01	77.03	53.25	36.66
21.81	[10]-gingerdione	14.46	16.29	15.29	10.01	11.21	18.84	16.58	10.76	12.64
Total:	**986.89**	**987.64**	**980.99**	**987.83**	**981.87**	**988.35**	**986.79**	**987.62**	**984.47**	

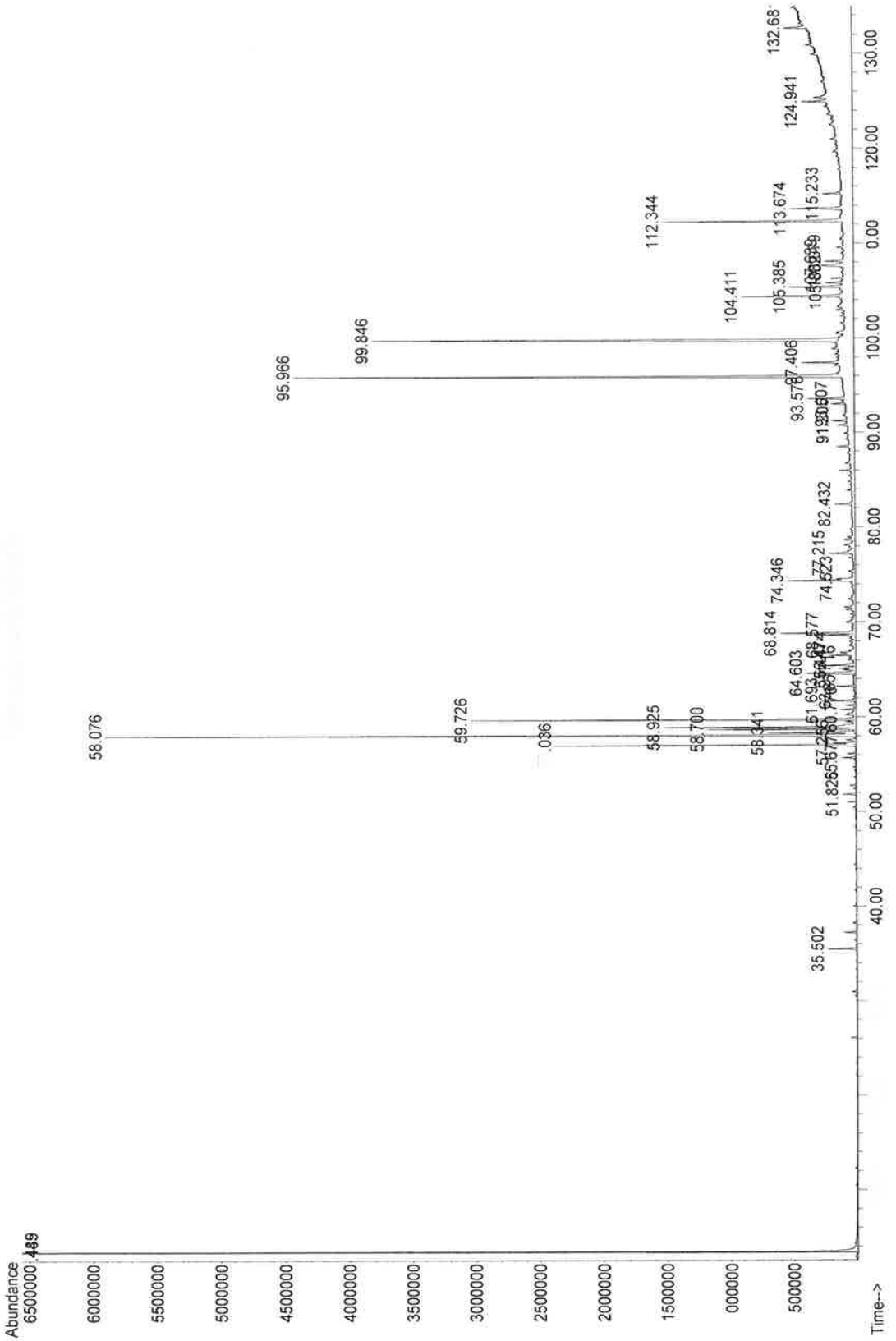
Appendix 3. GC of Acetone Extract Synthite Rajakumari Ginger



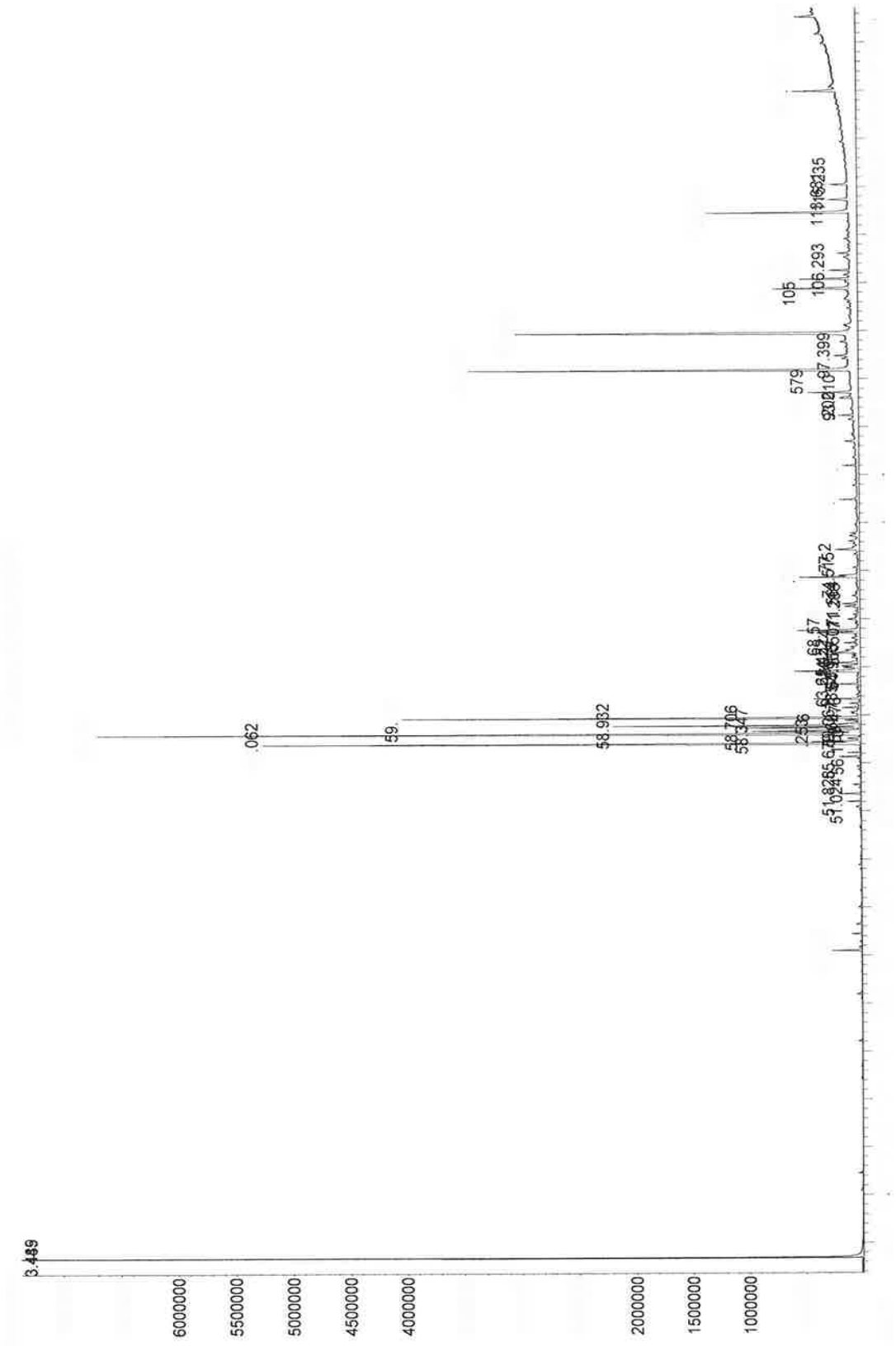
Appendix 4. GC of Acetone Extract Synthite Irutti Ginger



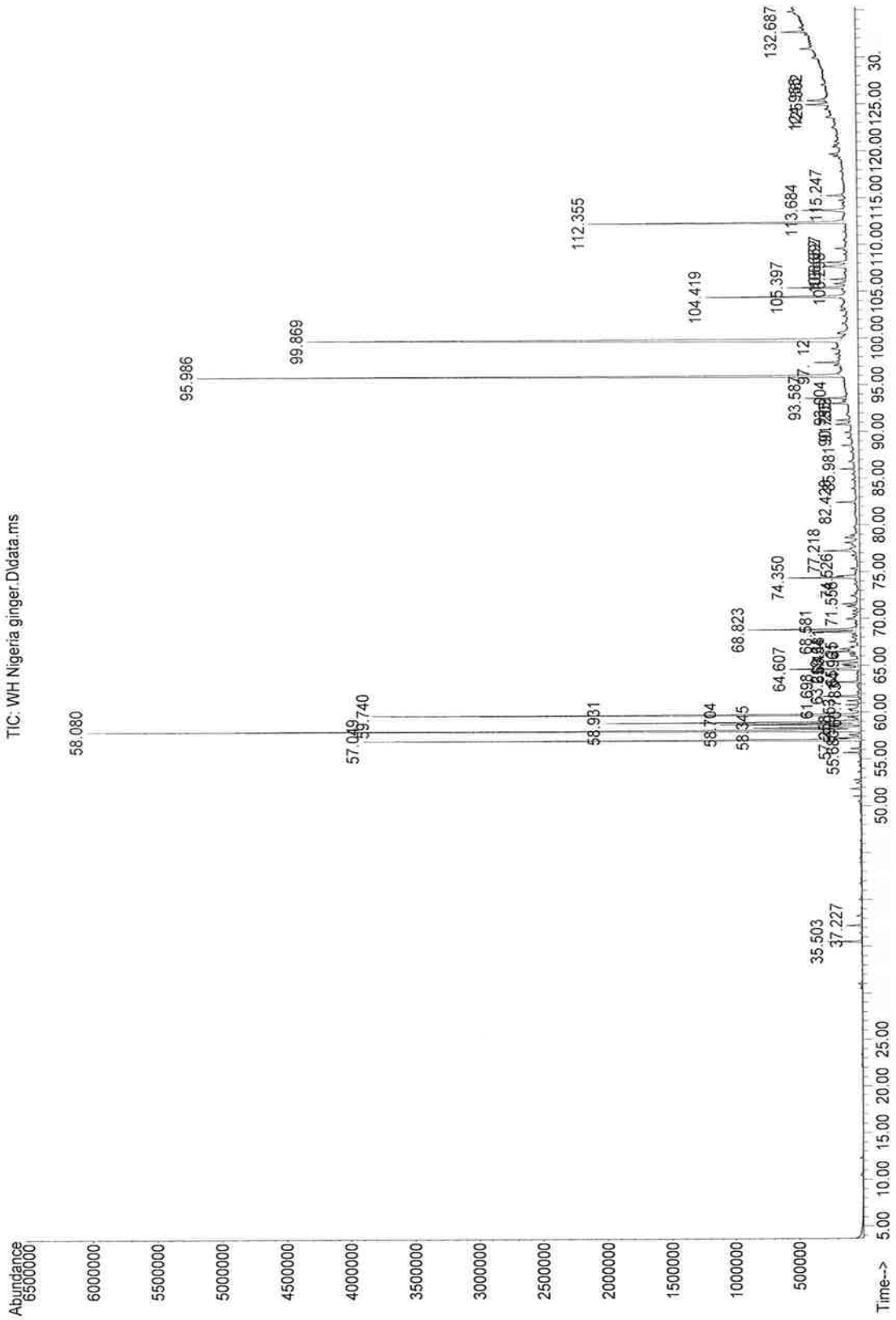
Appendix 5. GC of Acetone Extract Synthite Nigerian Ginger



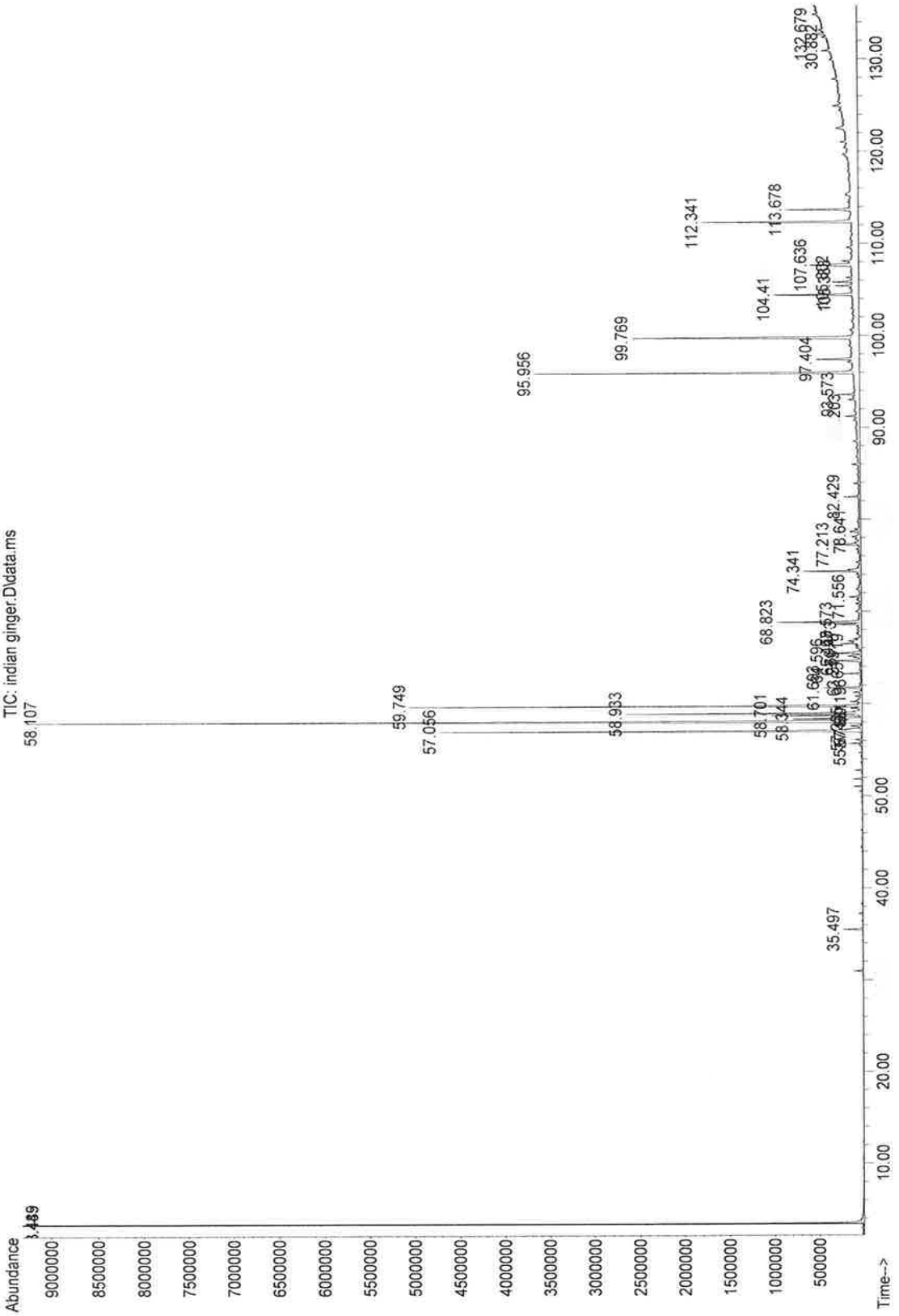
Appendix 6. GC of Acetone Extract Synthite Shimoga Ginger



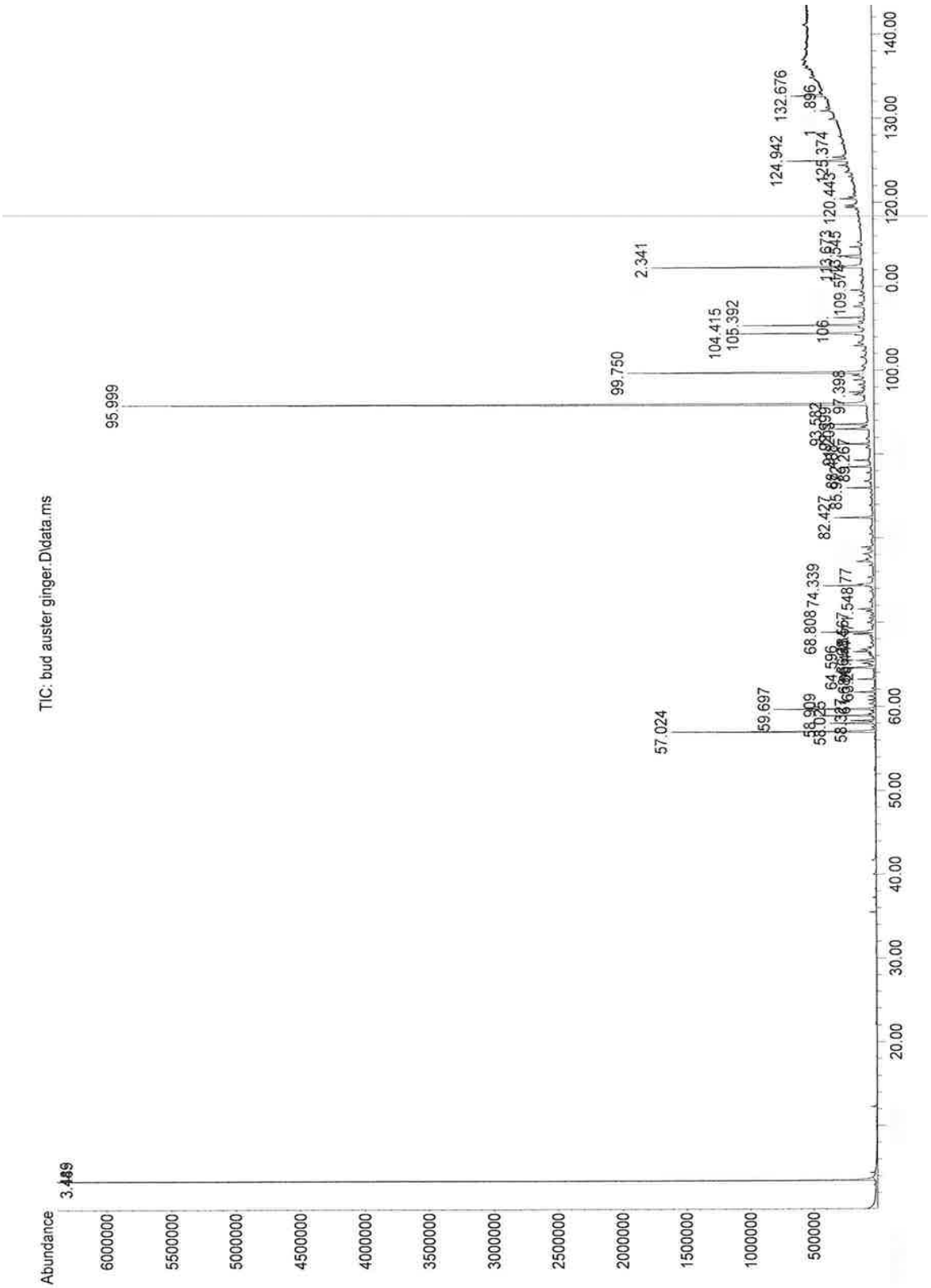
Appendix 7. GC of Acetone Extract Whole Herb Nigerian Ginger



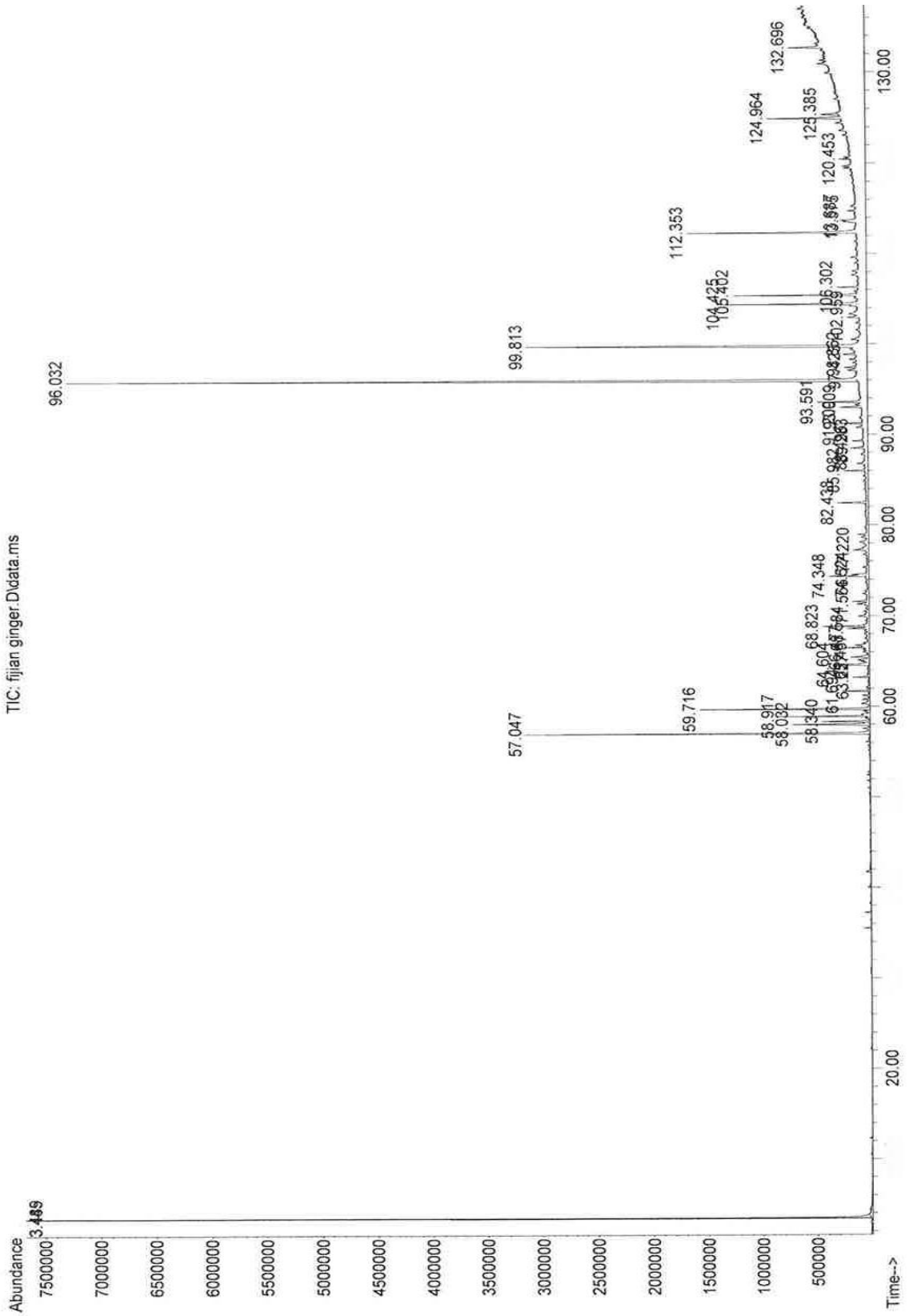
Appendix 8. GC of Acetone Extract Whole Herb Indian Ginger



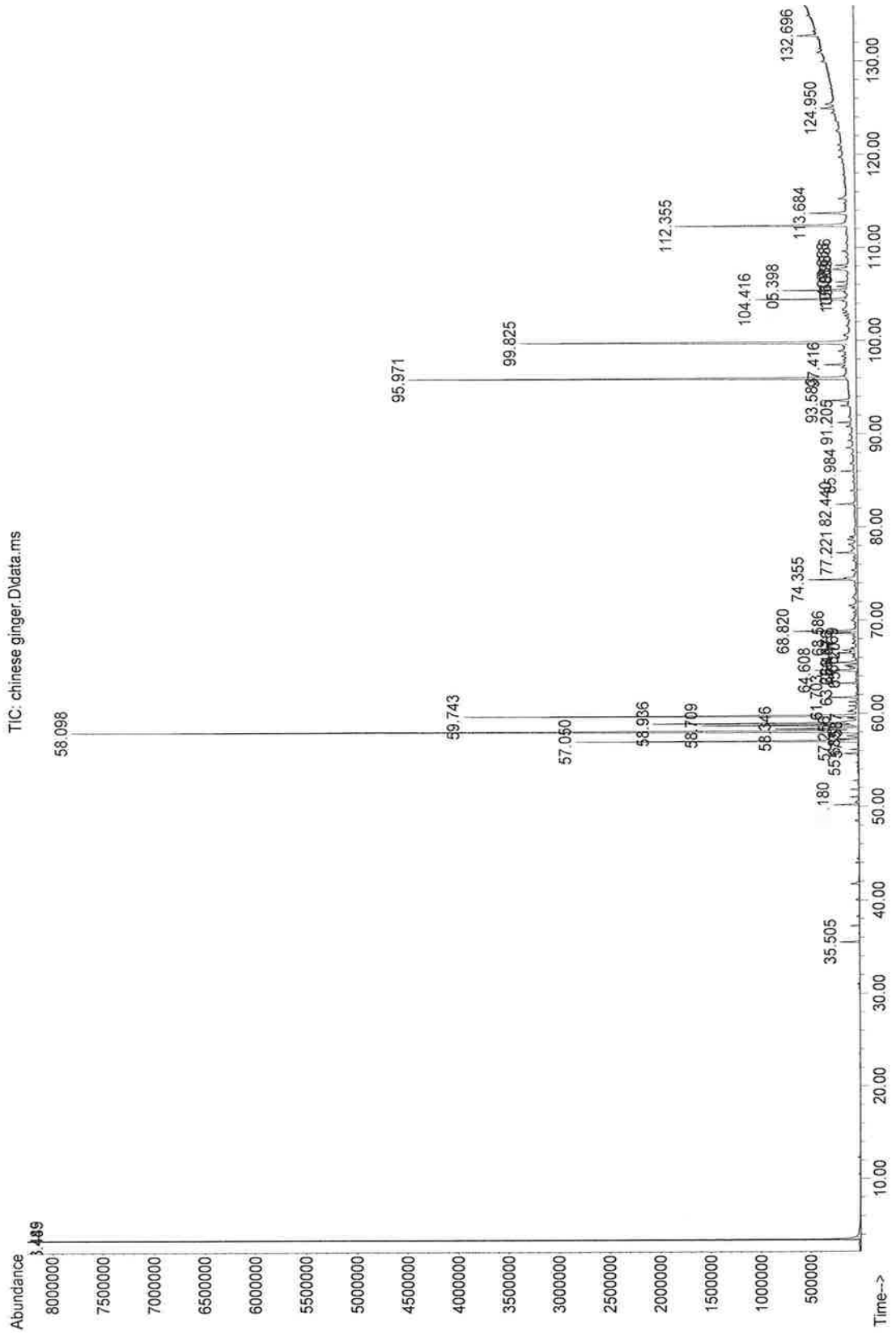
Appendix 9. GC of Acetone Extract Buderim Australian Ginger



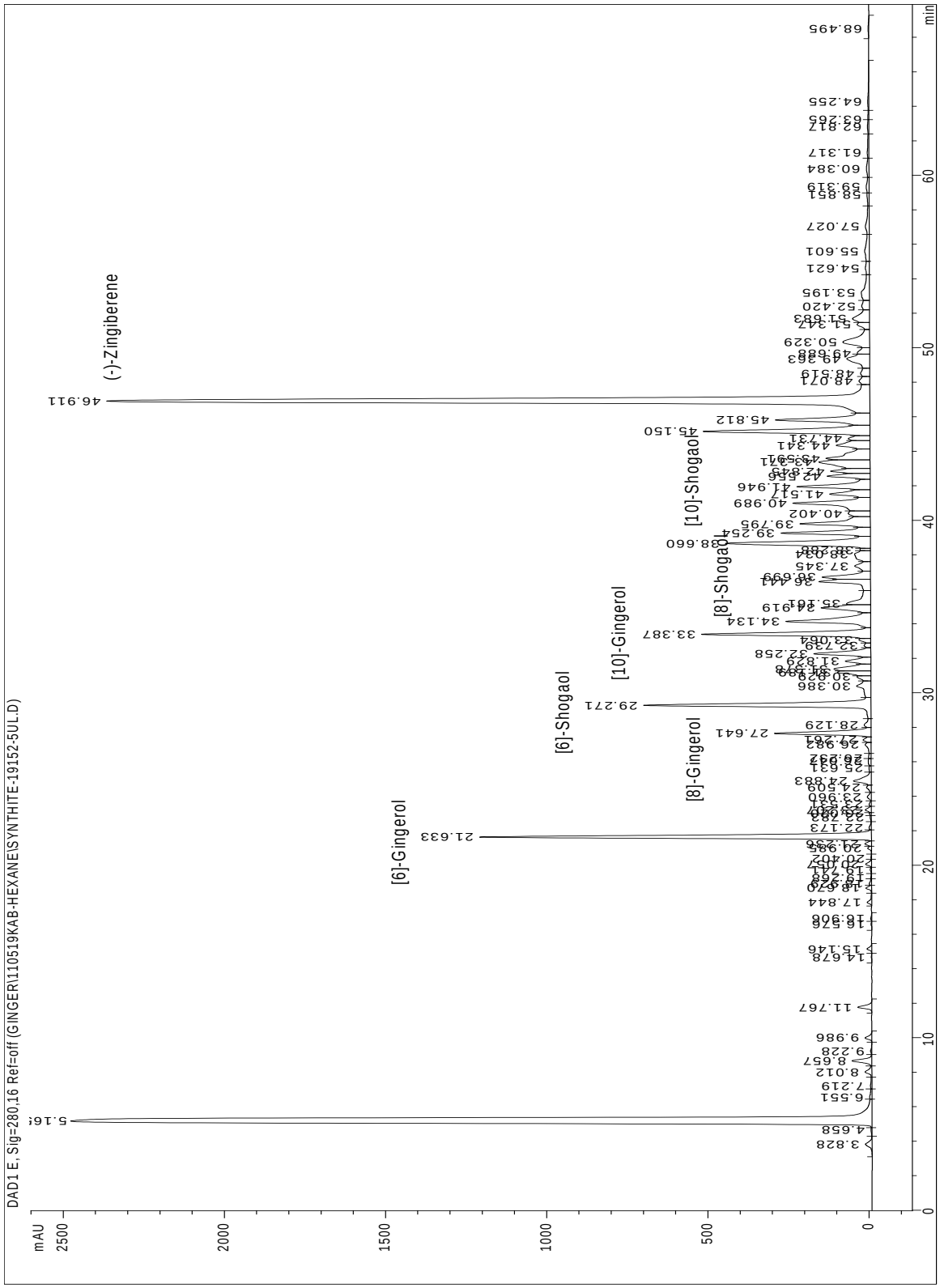
Appendix 10. GC of Acetone Extract Buderim Fijian Ginger



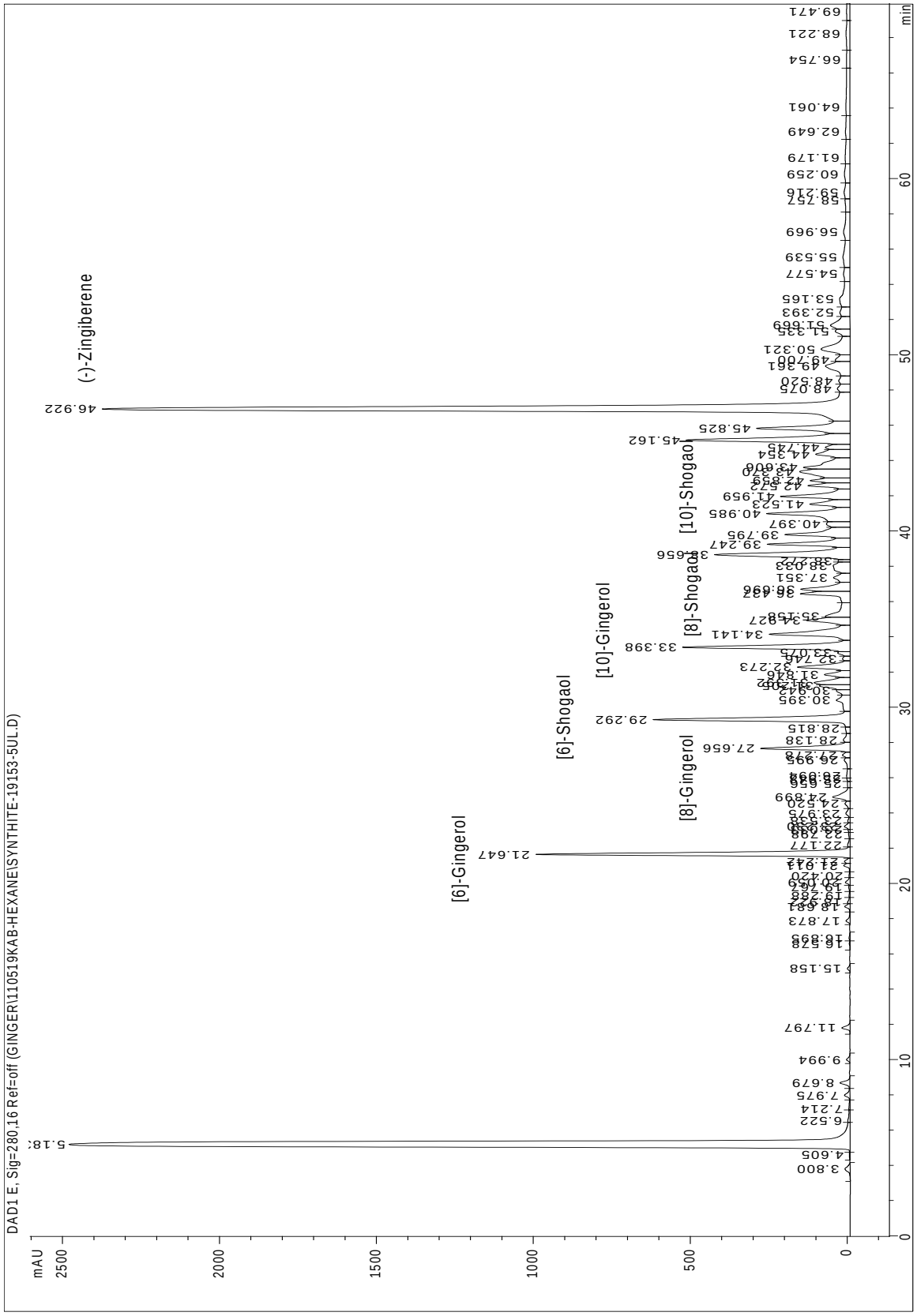
Appendix 11. GC of Acetone Extract PharmaChem Chinese Ginger



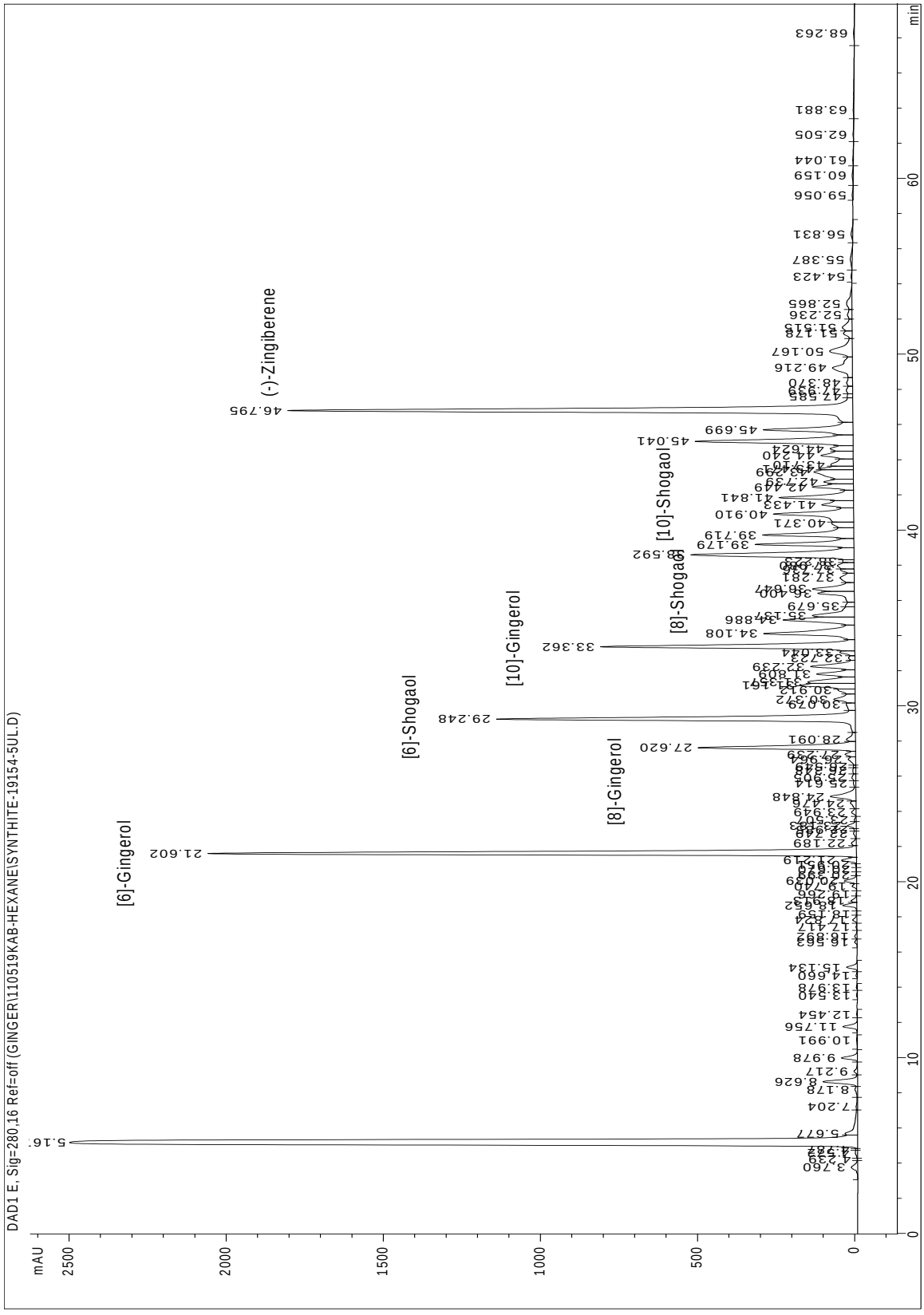
Appendix 12. HPLC of Hexane Extract Synthite Rajakumari Ginger



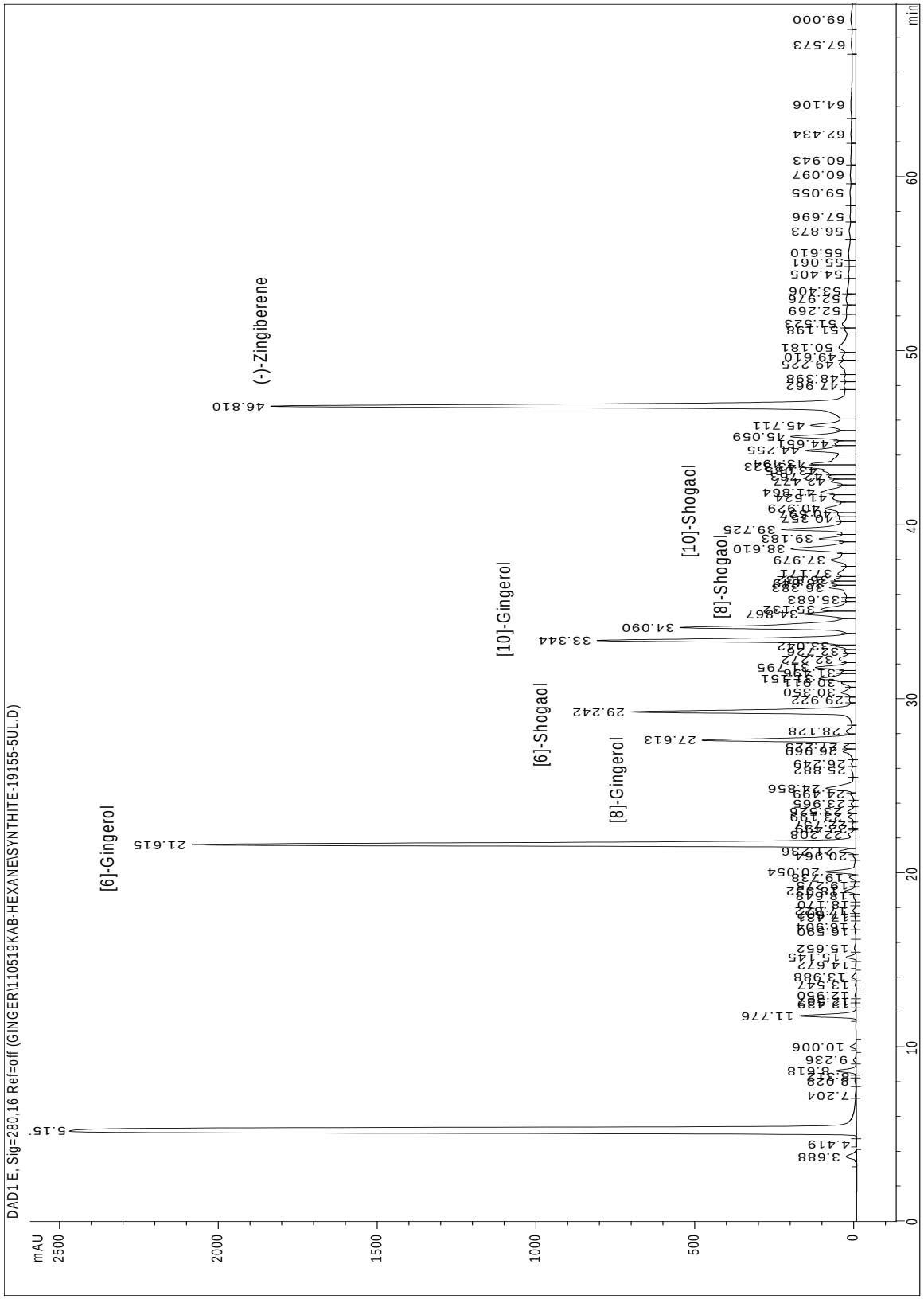
Appendix 13. HPLC of Hexane Extract Synthite Irutti Ginger



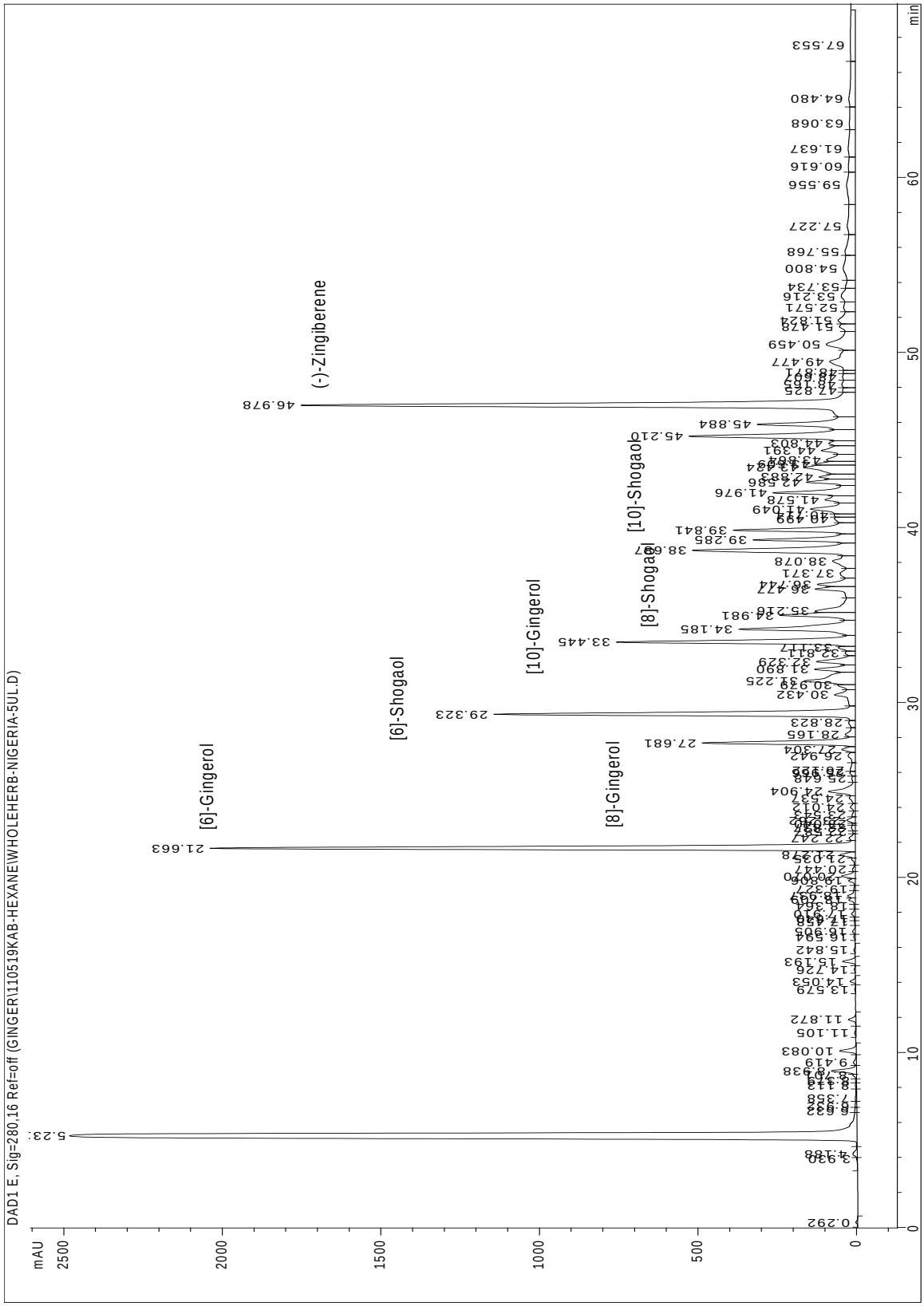
Appendix 14. HPLC of Hexane Extract Synthite Nigerian Ginger



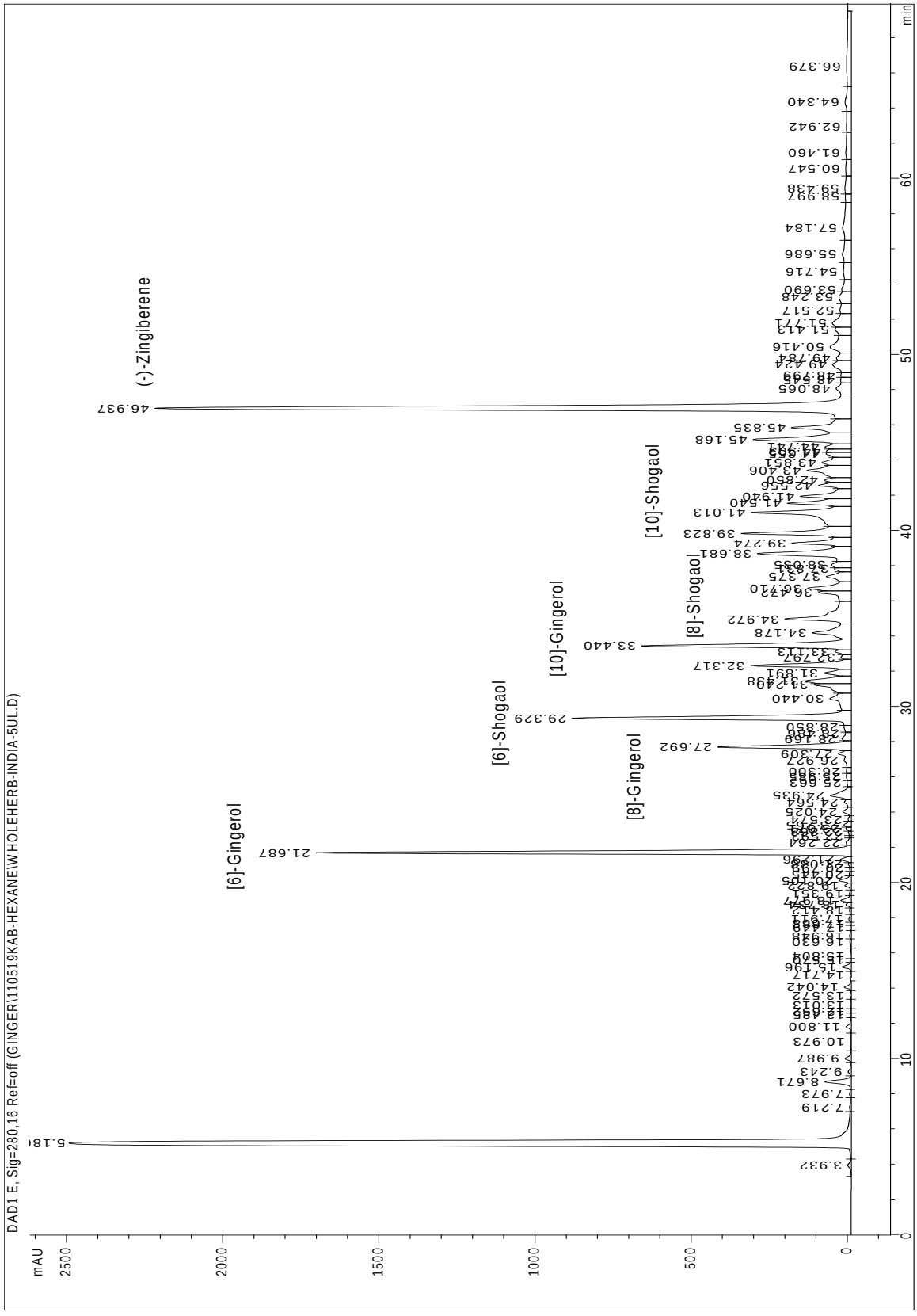
Appendix 15. HPLC of Hexane Extract Synthite Shimoga Ginger



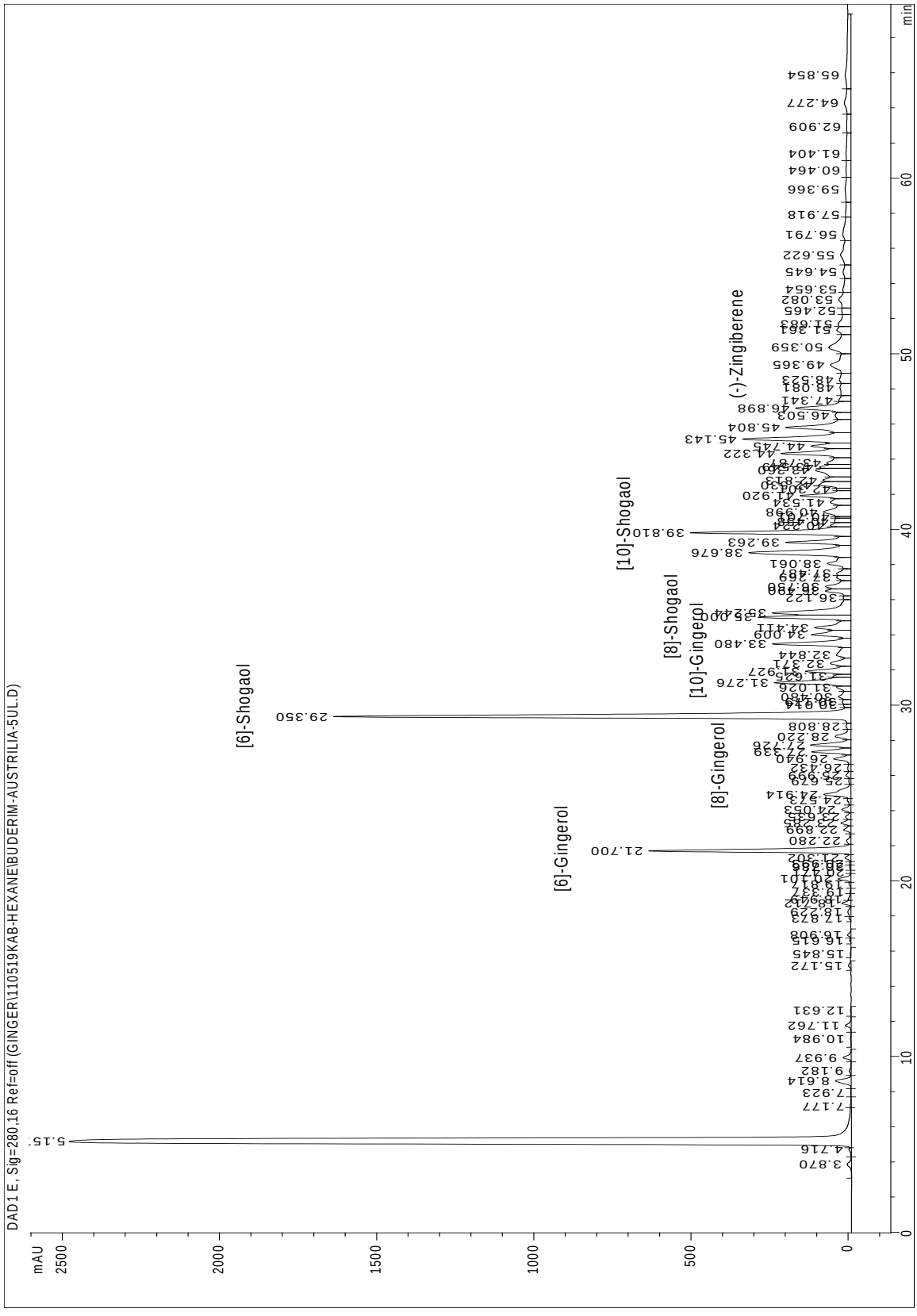
Appendix 16. HPLC of Hexane Extract Whole Herb Nigerian Ginger



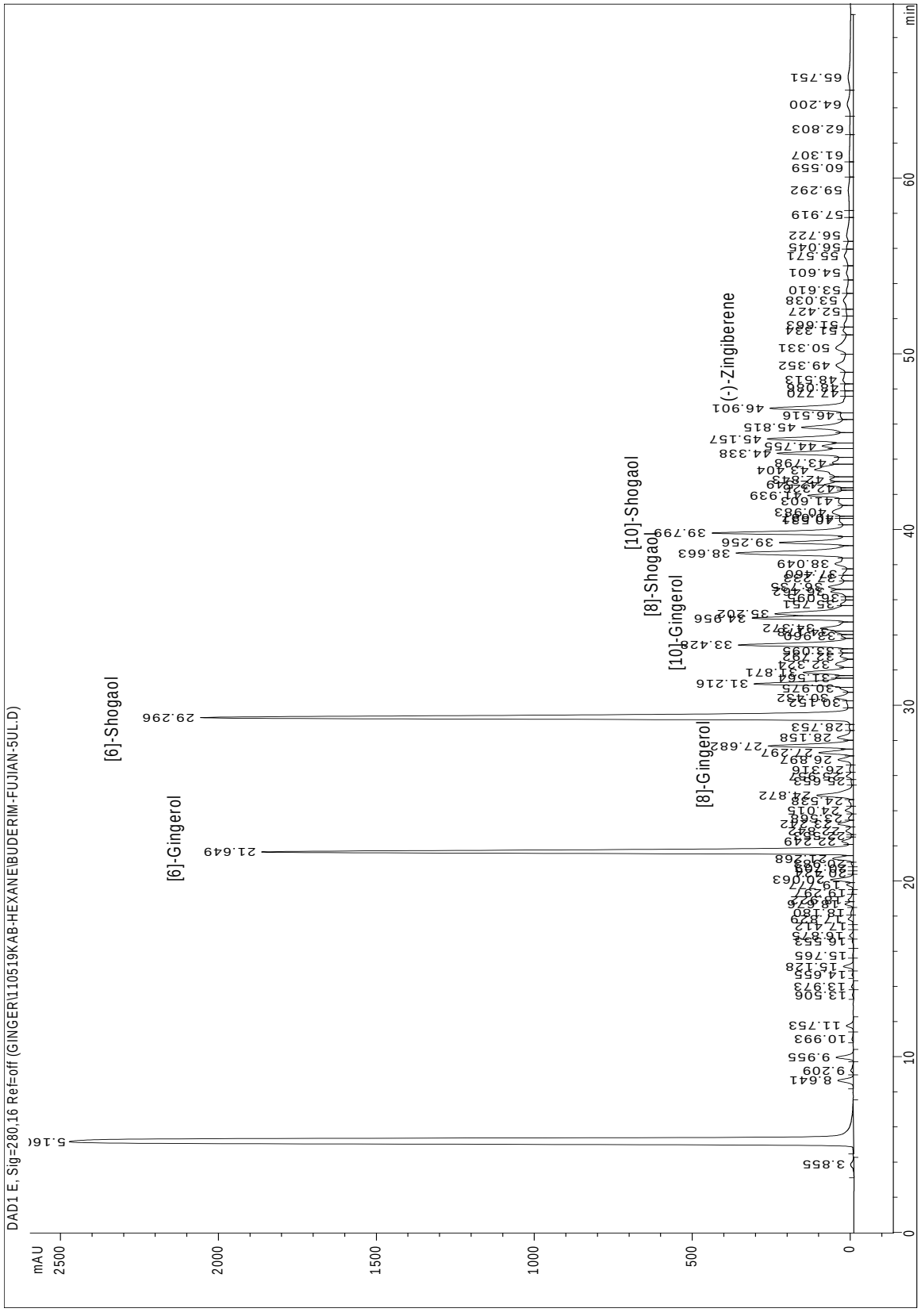
Appendix 17. HPLC of Hexane Extract Whole Herb Indian Ginger



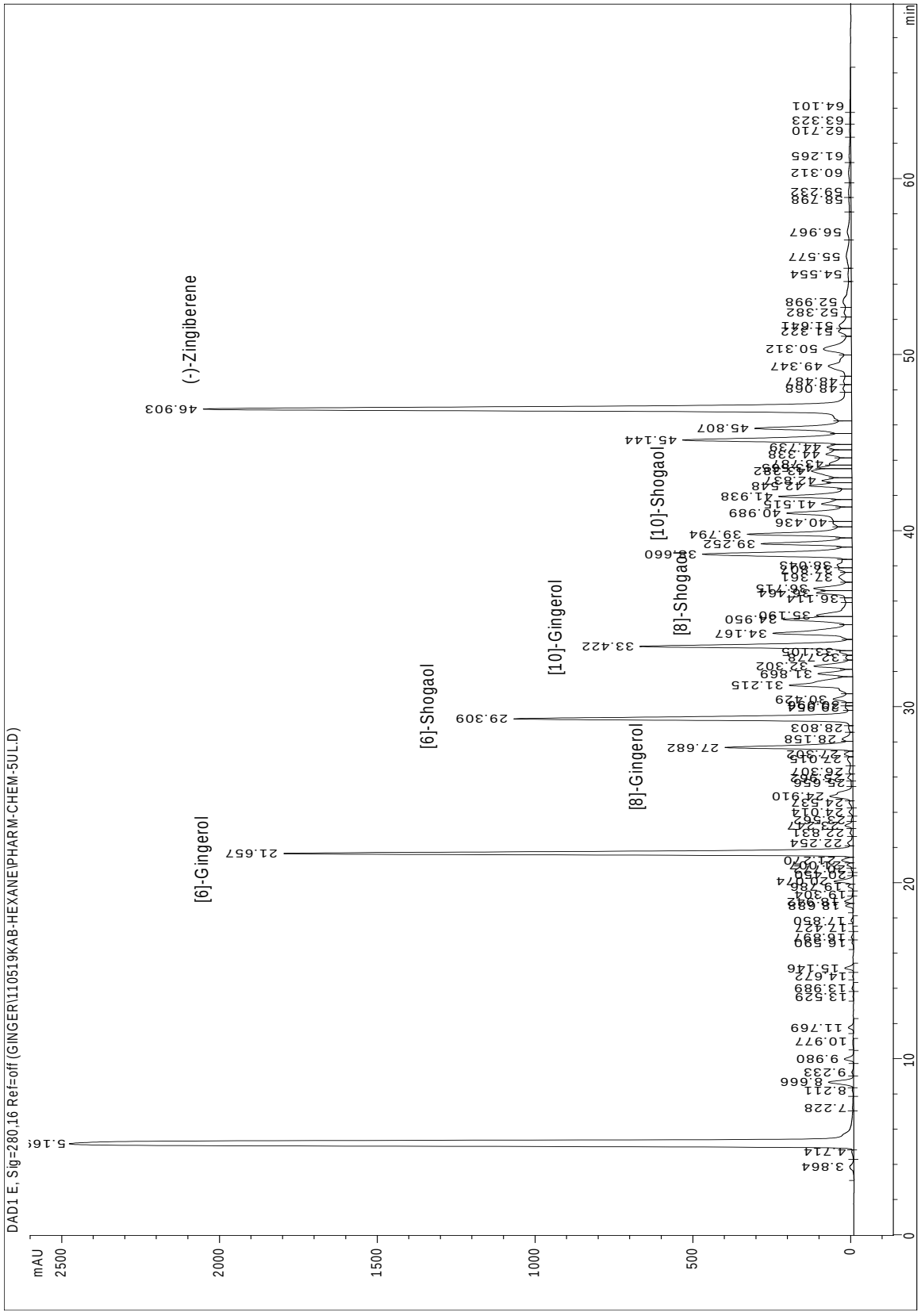
Appendix 18. HPLC of Hexane Extract Buderim Australian Ginger



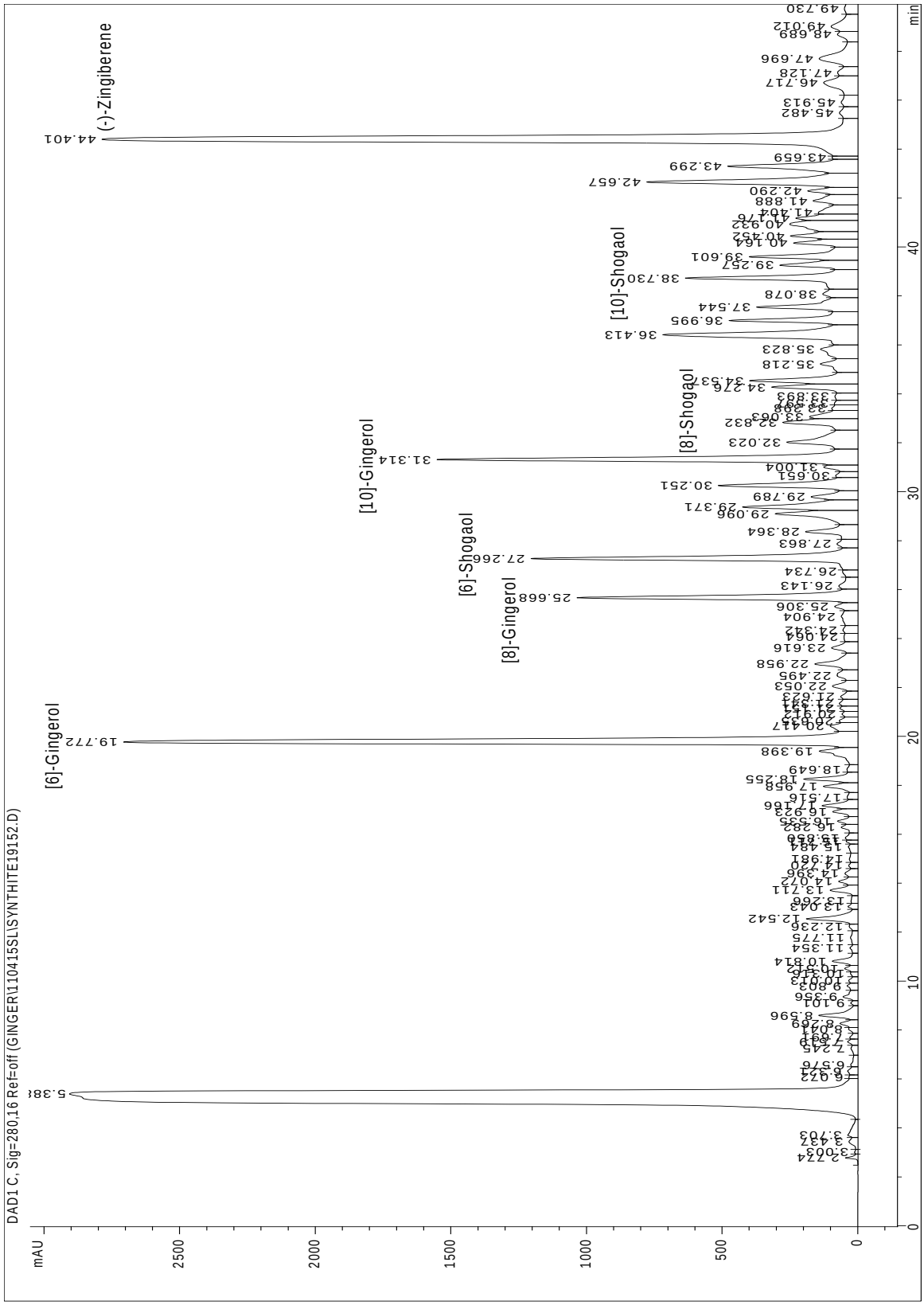
Appendix 19. HPLC of Hexane Extract Buderim Fijian Ginger



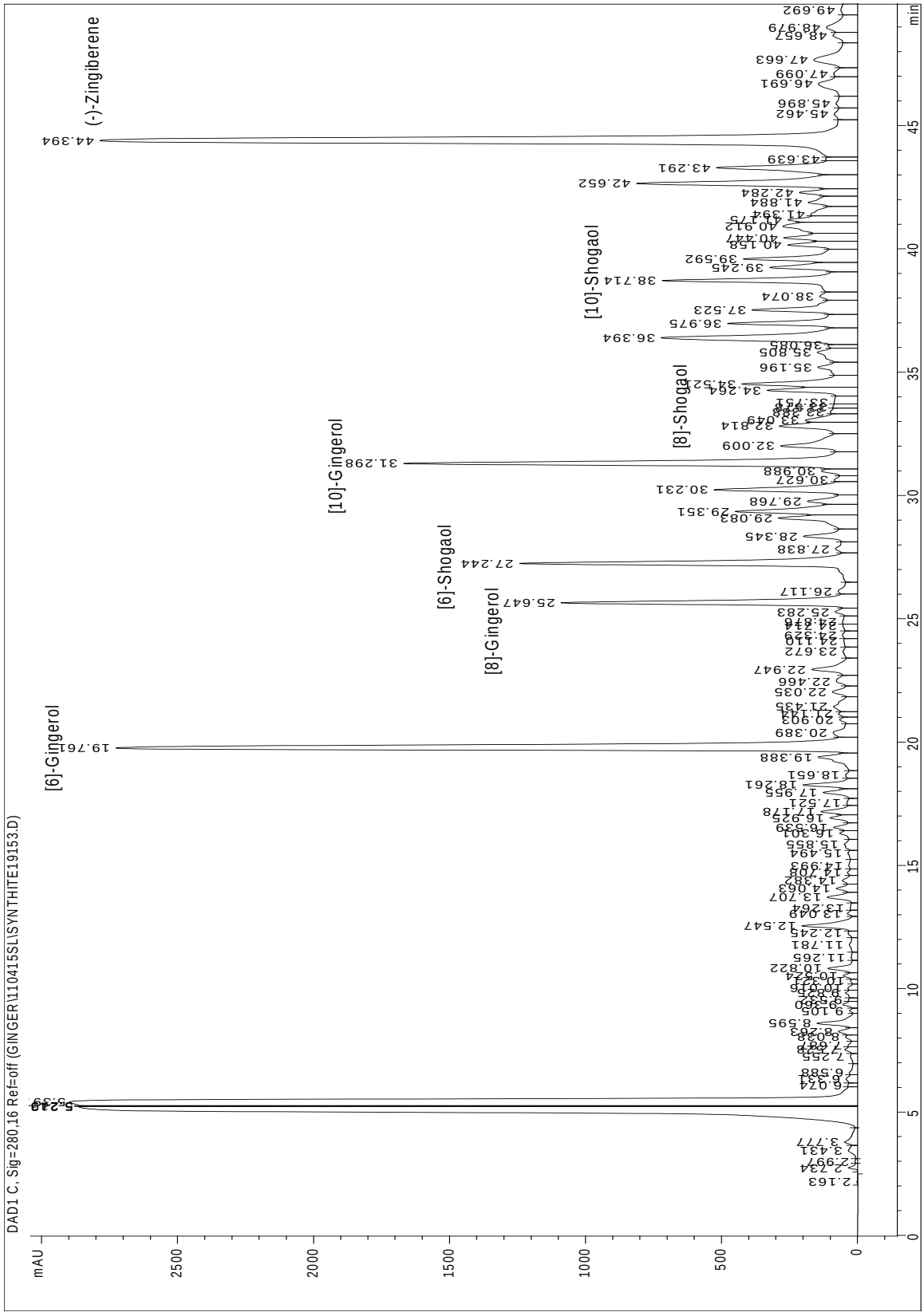
Appendix 20. HPLC of Hexane Extract PharmaChem Chinese Ginger



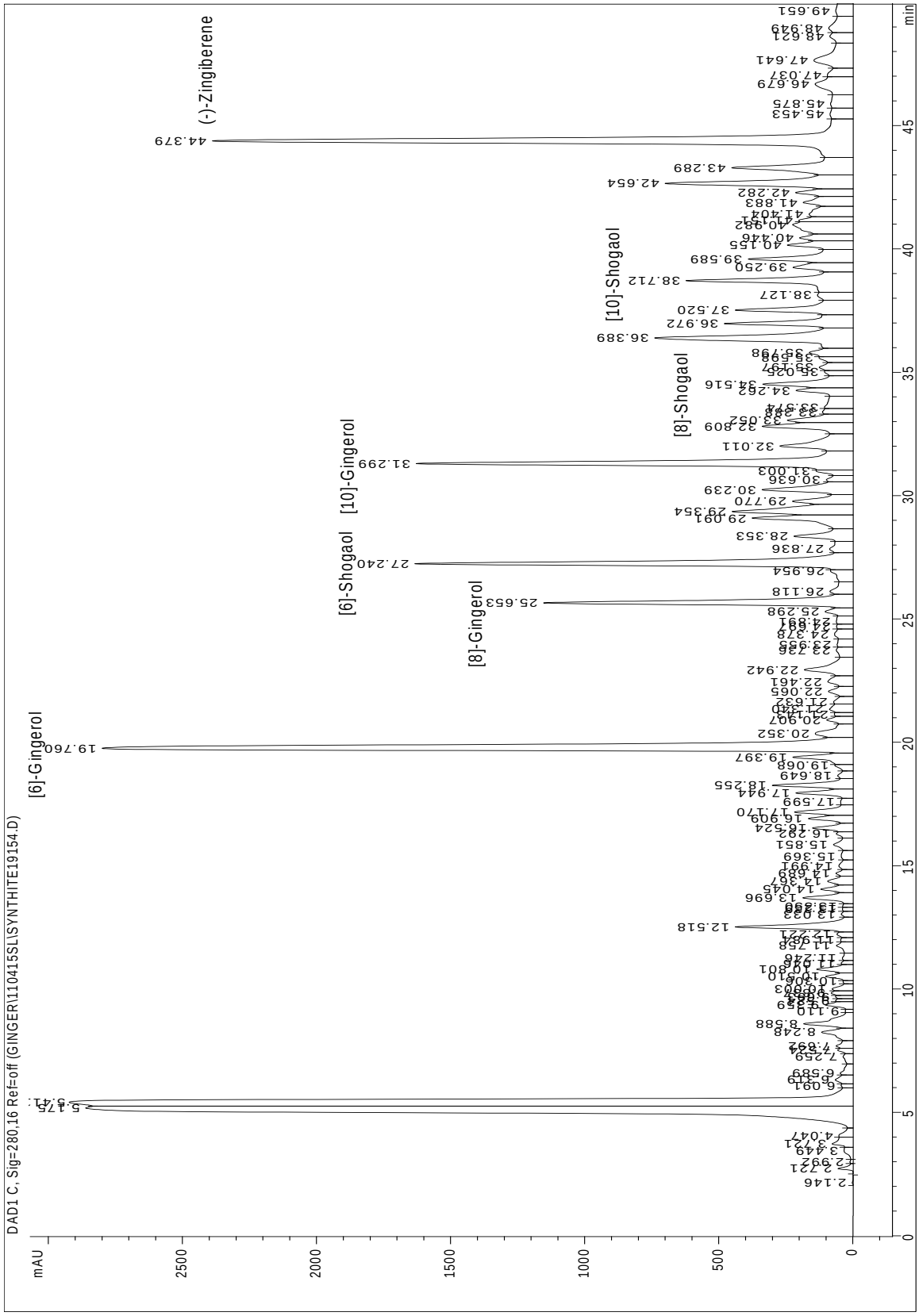
Appendix 21. HPLC of Acetone Extract Synthite Rajakumari Ginger



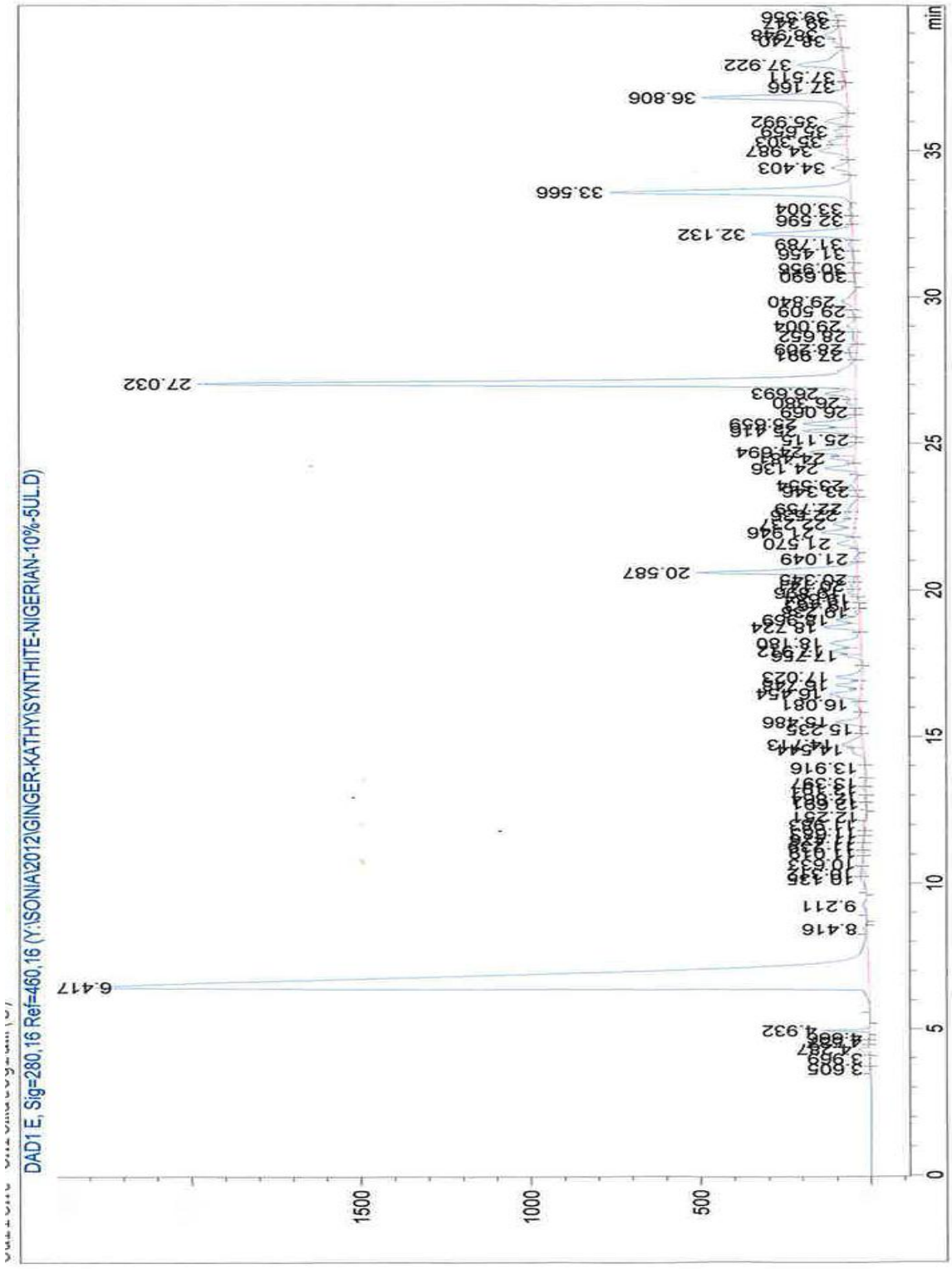
Appendix 22. HPLC of Acetone Extract Synthite Irutti Ginger



Appendix 23a. HPLC of Acetone Extract Synthite Nigerian Ginger

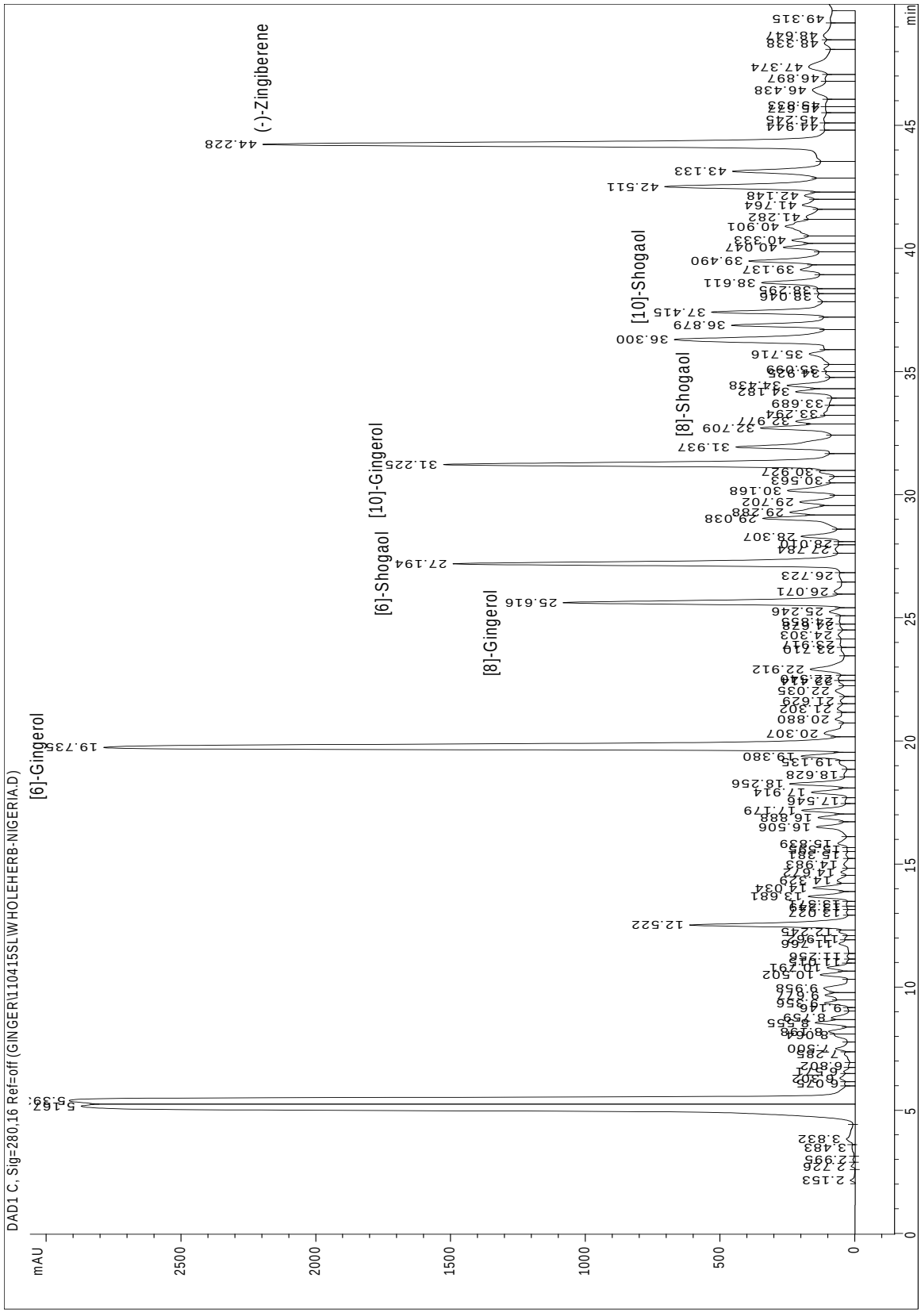


Appendix 23b. HPLC of Hexane Washed, Acetone Extract Synthite Nigerian Ginger

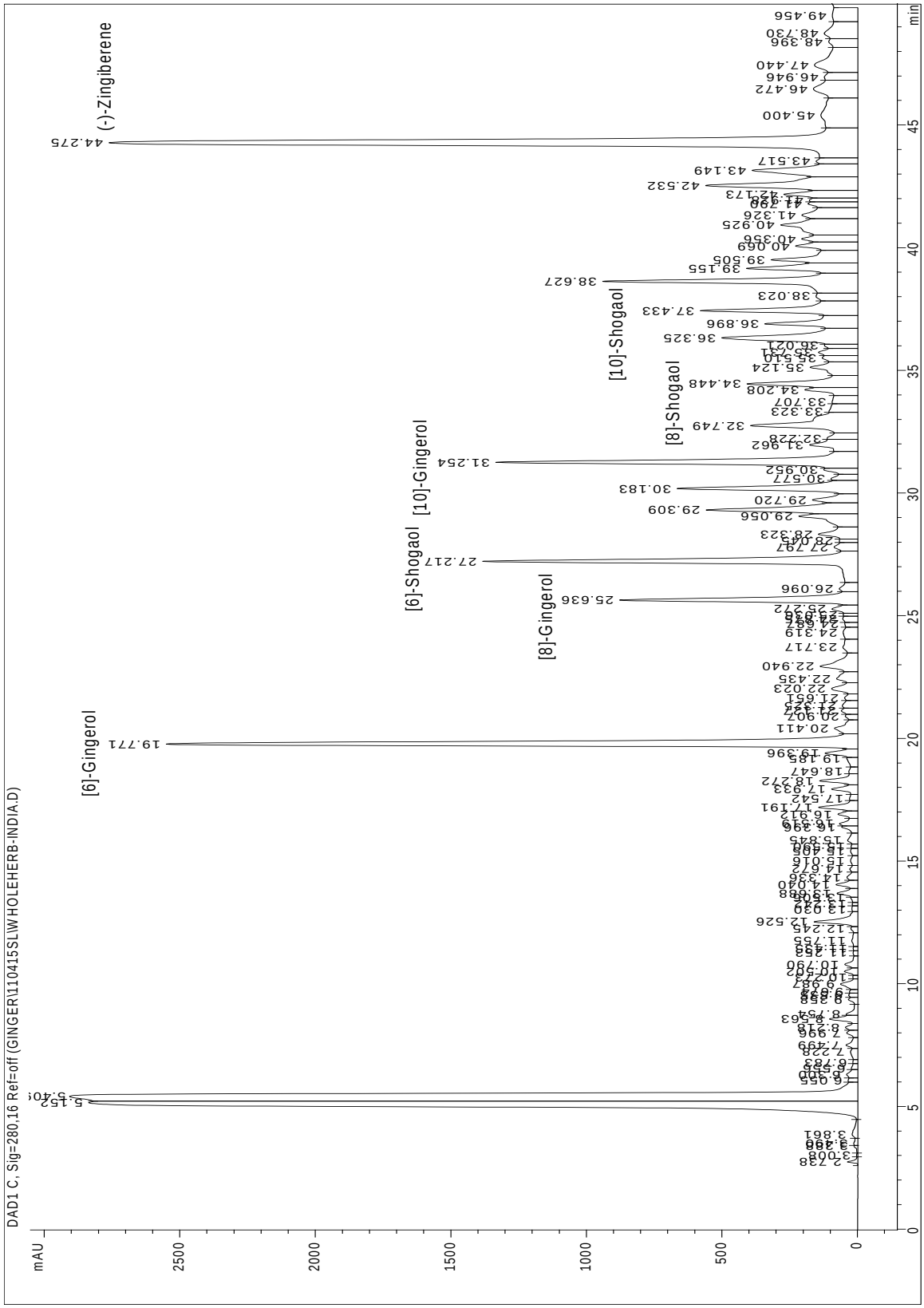


Appendix 24. HPLC of Acetone Extract Synthite Shimoga Ginger

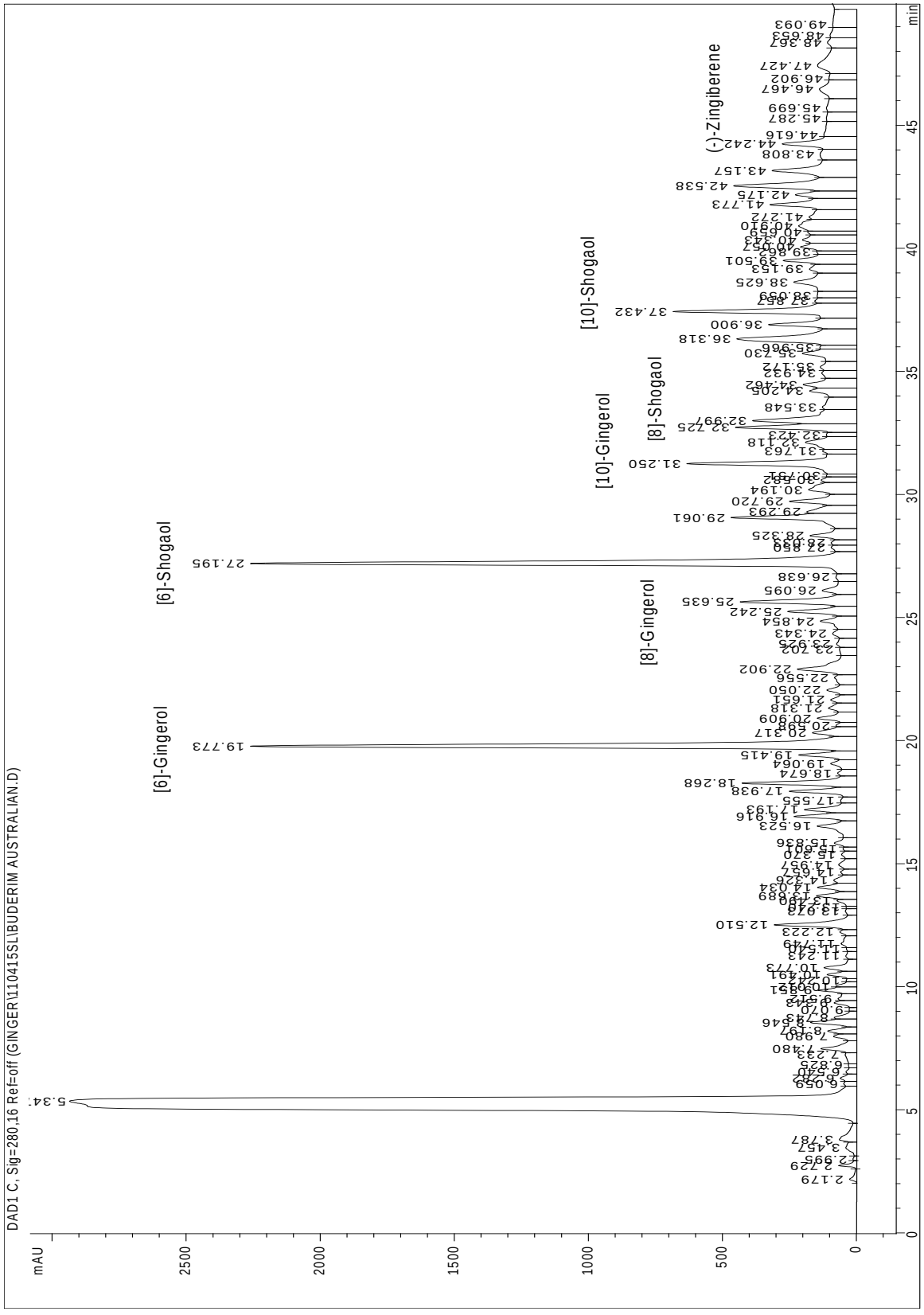
Appendix 25. HPLC of Acetone Extract Whole Herb Nigerian Ginger



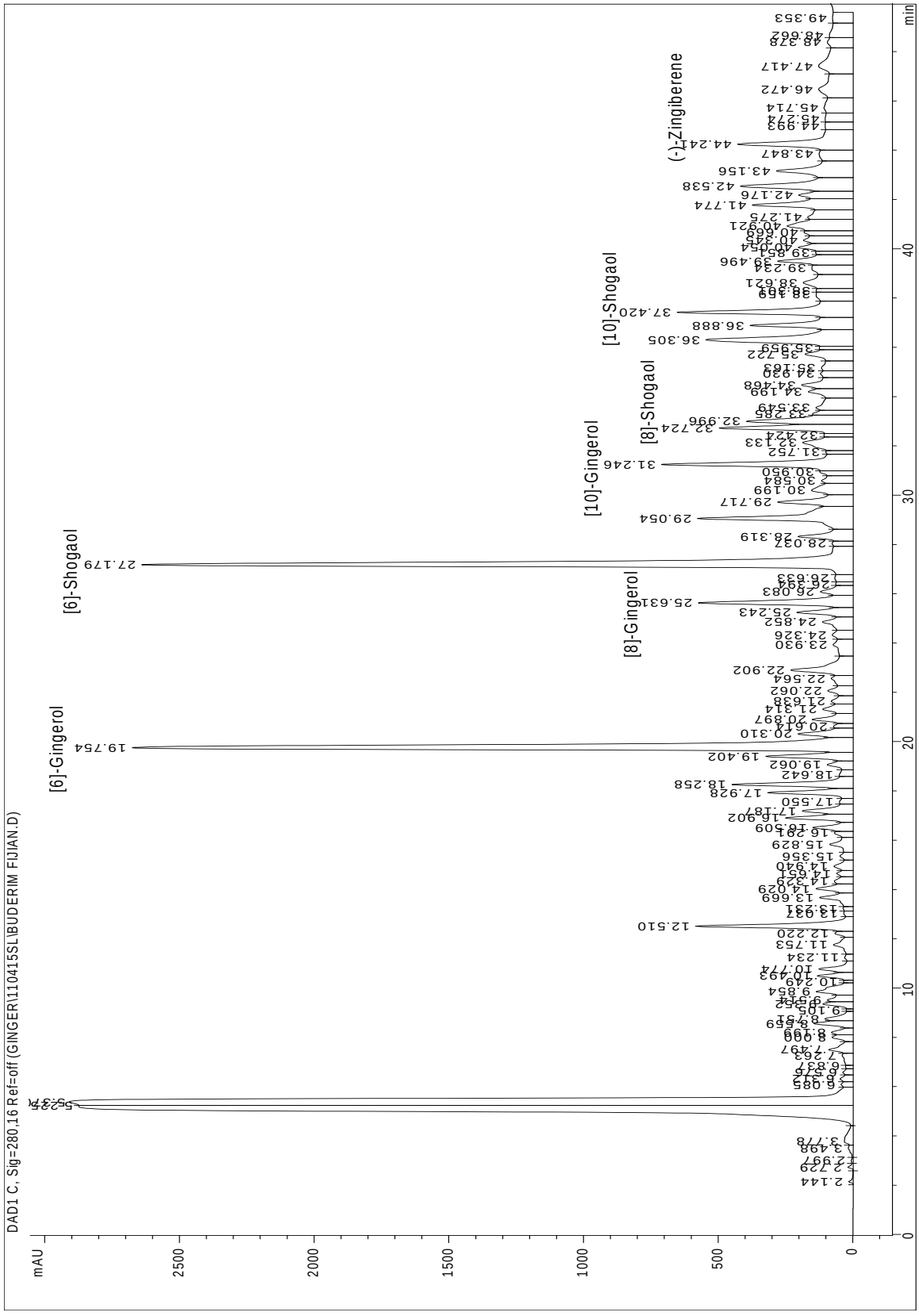
Appendix 26. HPLC of Acetone Extract Whole Herb Indian Ginger



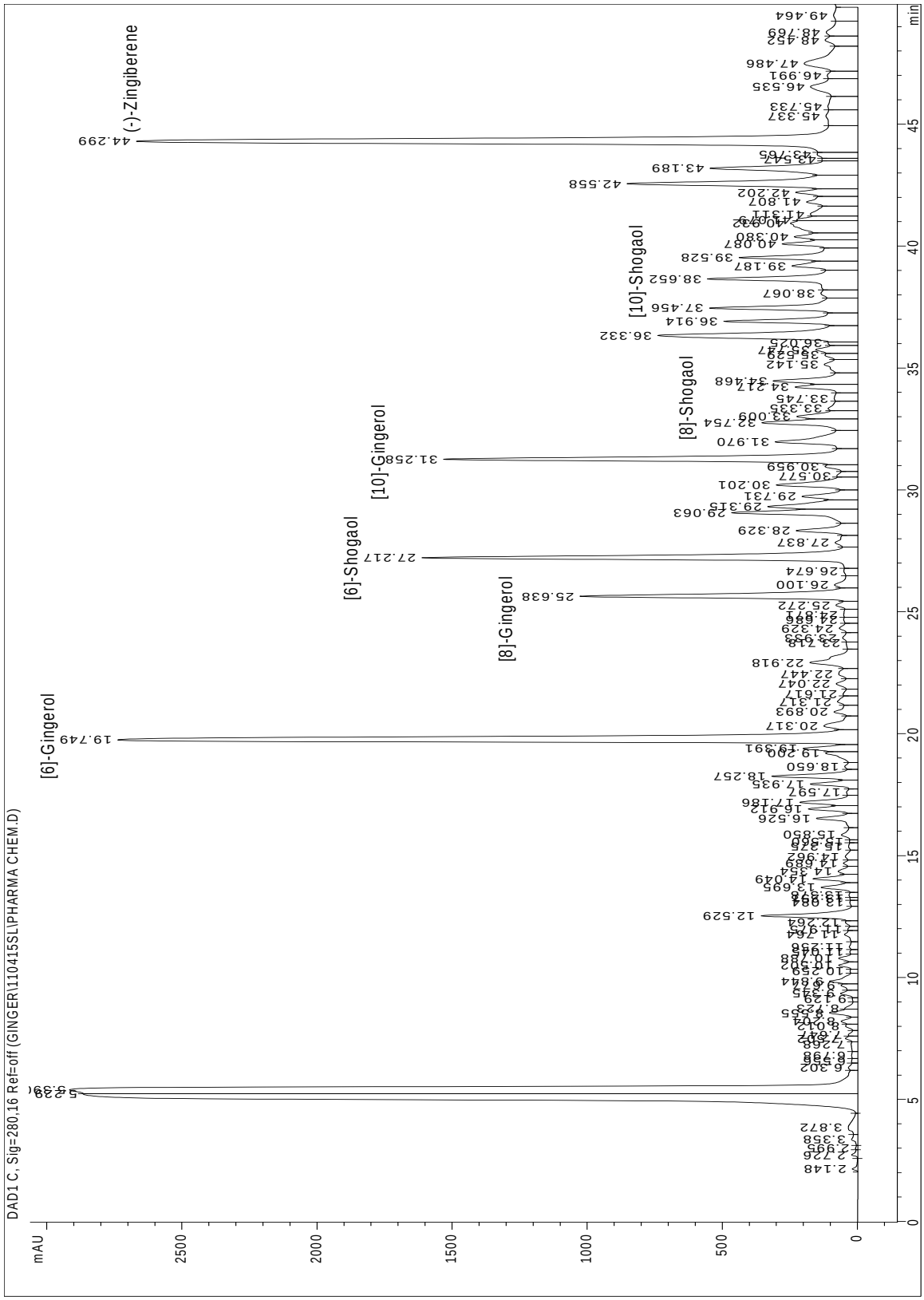
Appendix 27. HPLC of Acetone Extract Buderim Australian Ginger



Appendix 28. HPLC of Acetone Extract Buderim Fijian Ginger



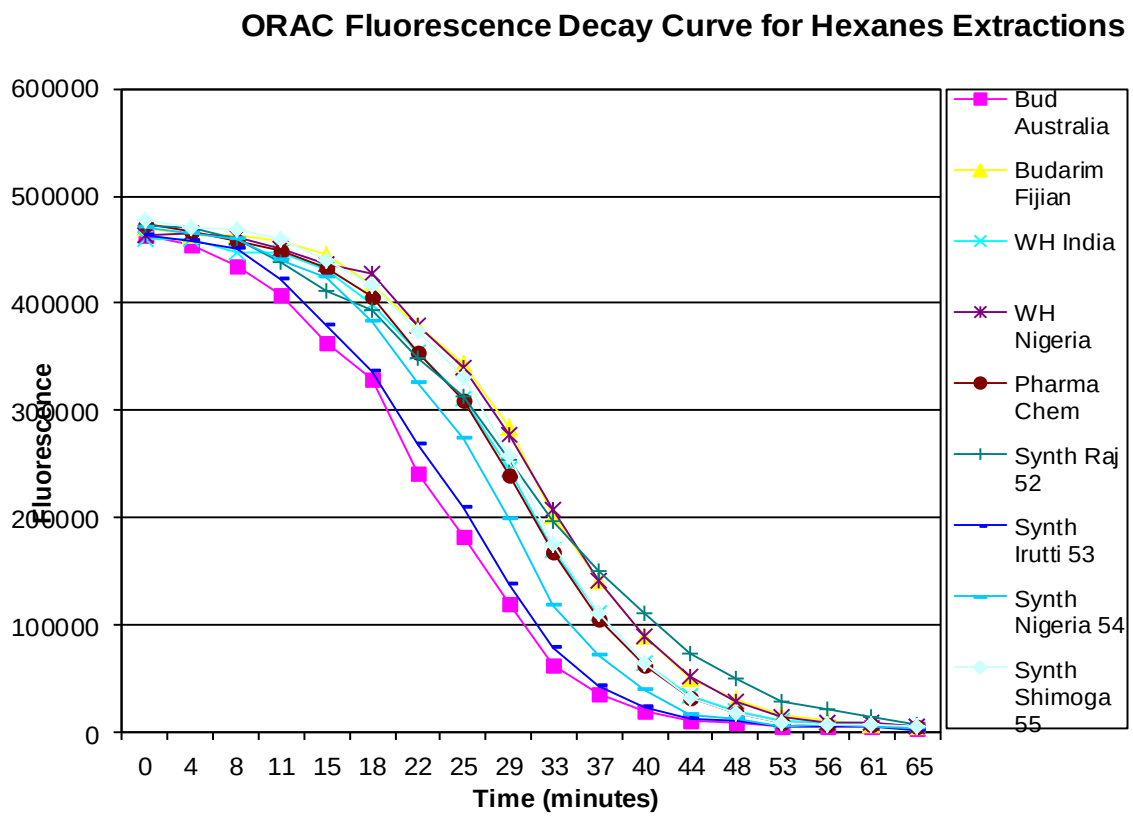
Appendix 29. HPLC of Acetone Extract PharmaChem Chinese Ginger



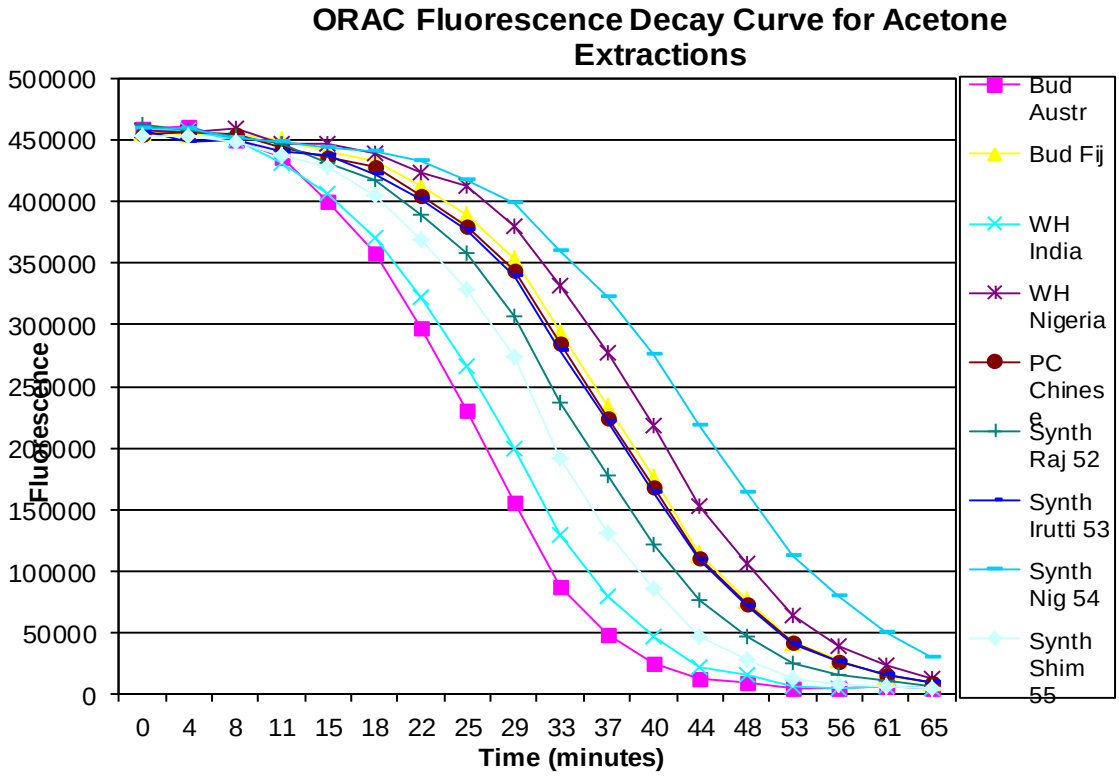
Appendix 30: Peak Area Summation of Gingerols and Shogaols from HPLC

Peak Area	Synthite Rajakumari	Synthite Irutti	Synthite Nigerian	Synthite Shimoga	Whole Herb Nigerian	Whole Herb Indian	Buderim Australian	Buderim Fijian	Pharma Chem Chinese
Hexane Gingerol Area:	25026.75	22440.68	43529.70	44371.15	40651.50	34074.51	11576.48	31089.48	36202.09
Hexane Shogaol Area:	13372.09	11968.53	21048.17	13346.46	20820.40	18169.87	30446.72	37906.87	20272.00
Hexane Total Area:	38398.84	34409.21	64577.87	57717.60	61471.90	52244.38	42023.20	68996.35	56474.09
Acetone Gingerol Area:	64951.45	68293.89	74292.32	61574.47	69831.28	55195.54	34296.34	48399.82	67552.80
Acetone Shogaol Area:	18856.25	19849.65	25356.26	17988.23	23919.88	25050.72	34847.26	42712.35	25962.25
Acetone Total Area:	83807.70	88143.54	99648.58	79562.70	93751.15	80246.26	69143.60	91112.17	93515.06

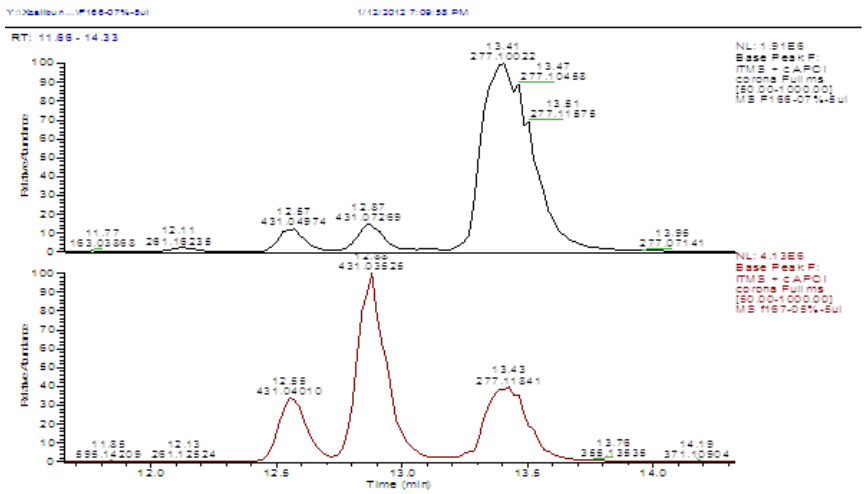
Appendix 31. Hexane Fluorescence Decay Curve



Appendix 32. Acetone Fluorescence Decay Curve

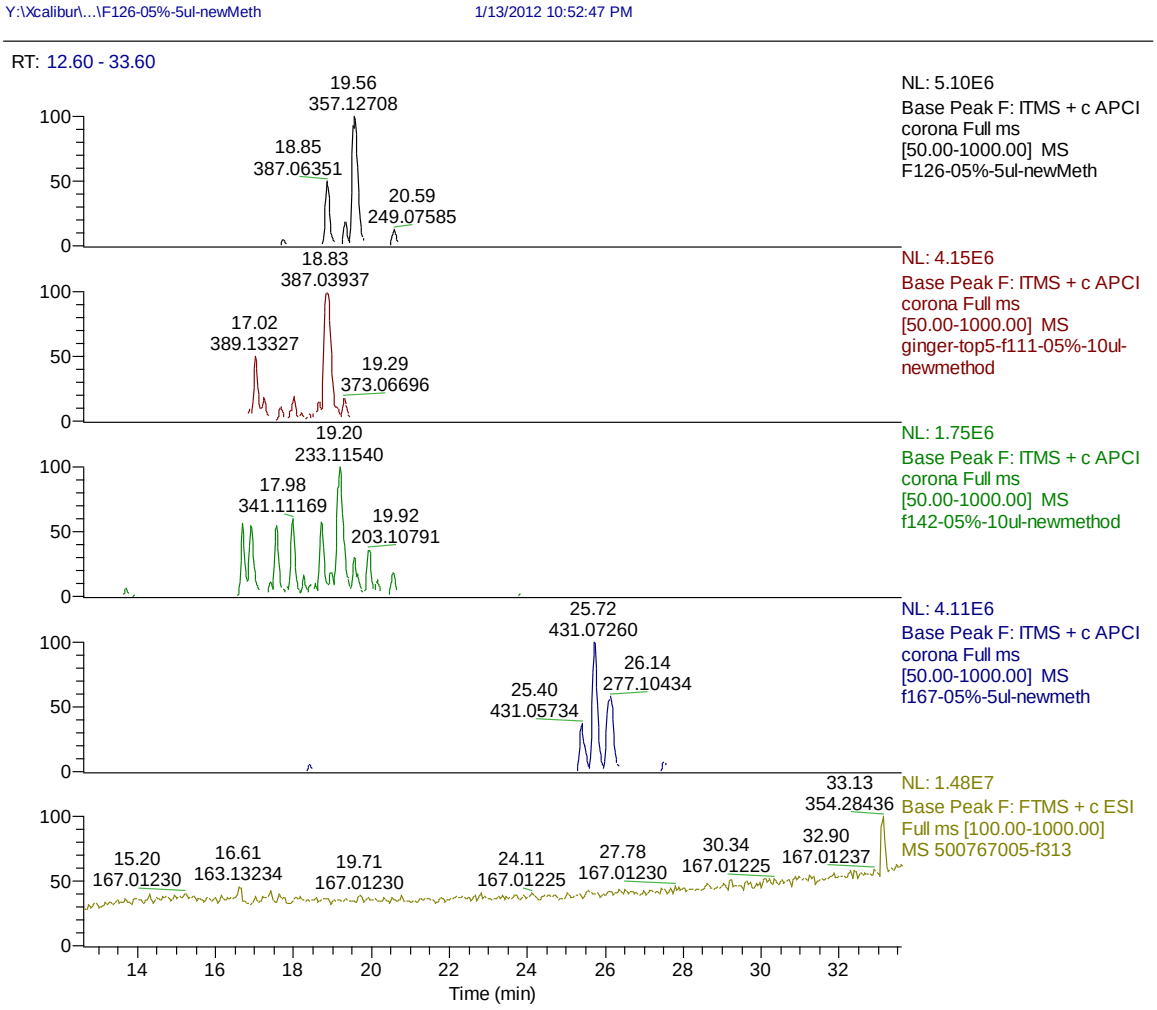


Appendix 33. The LC/MS Chromatograms of F166 and F167



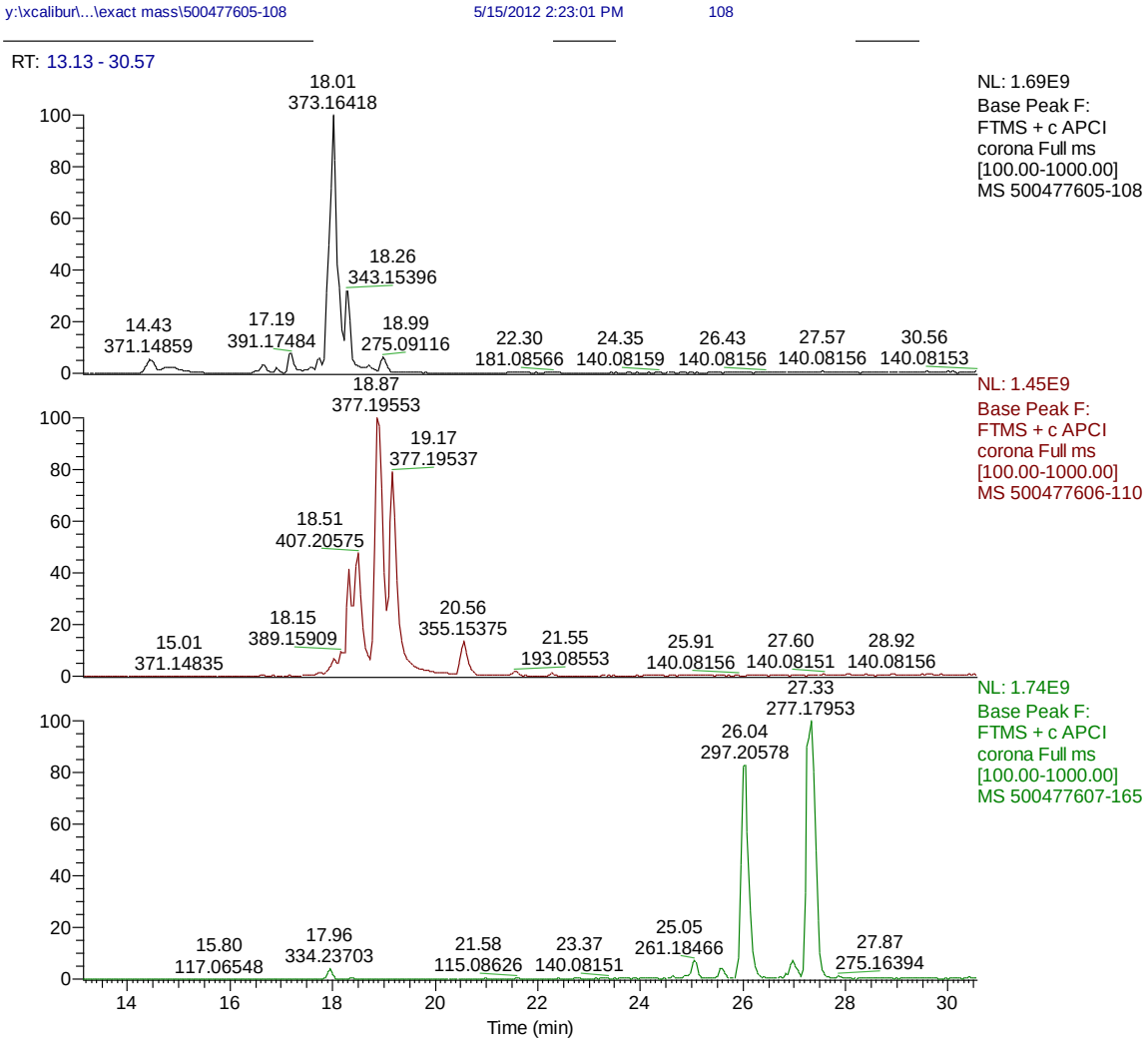
*F166, [6]-Gingerol (top) and F167 (bottom)

Appendix 34. The LC/MS Chromatograms of F126, F111, F142 and F167

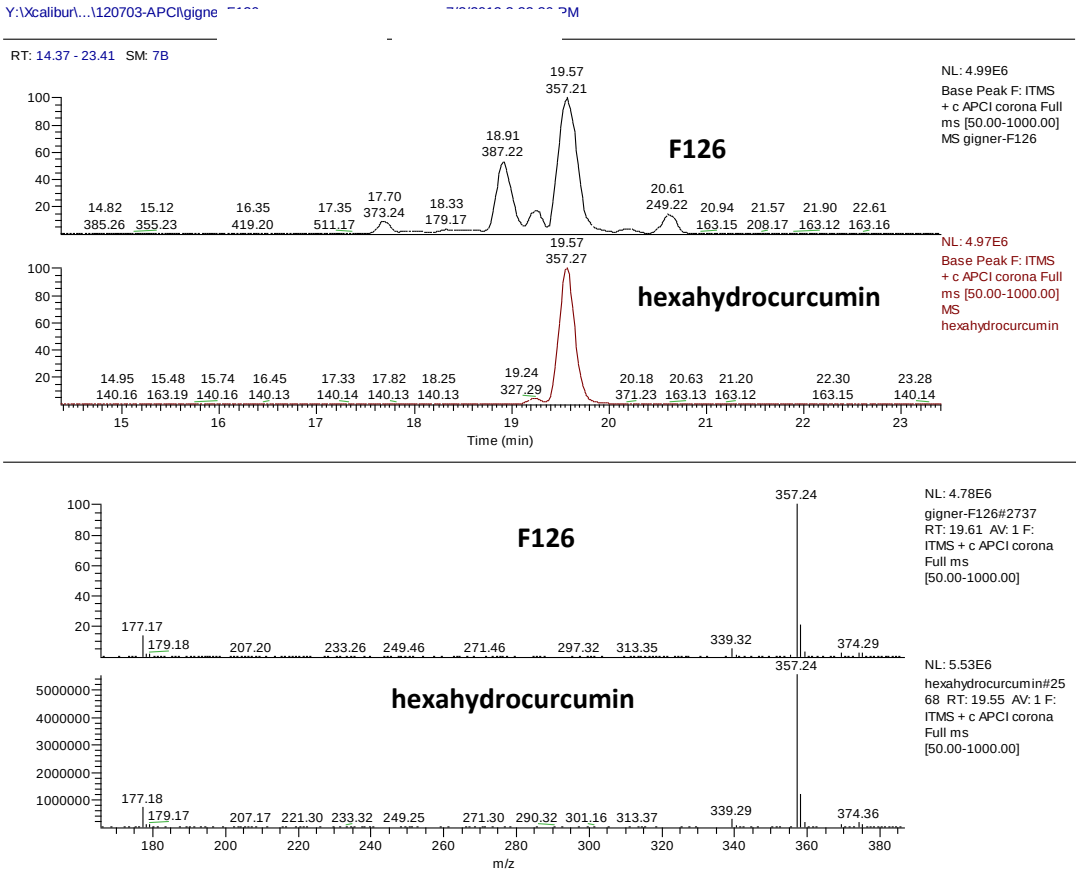


*F313 was not determined

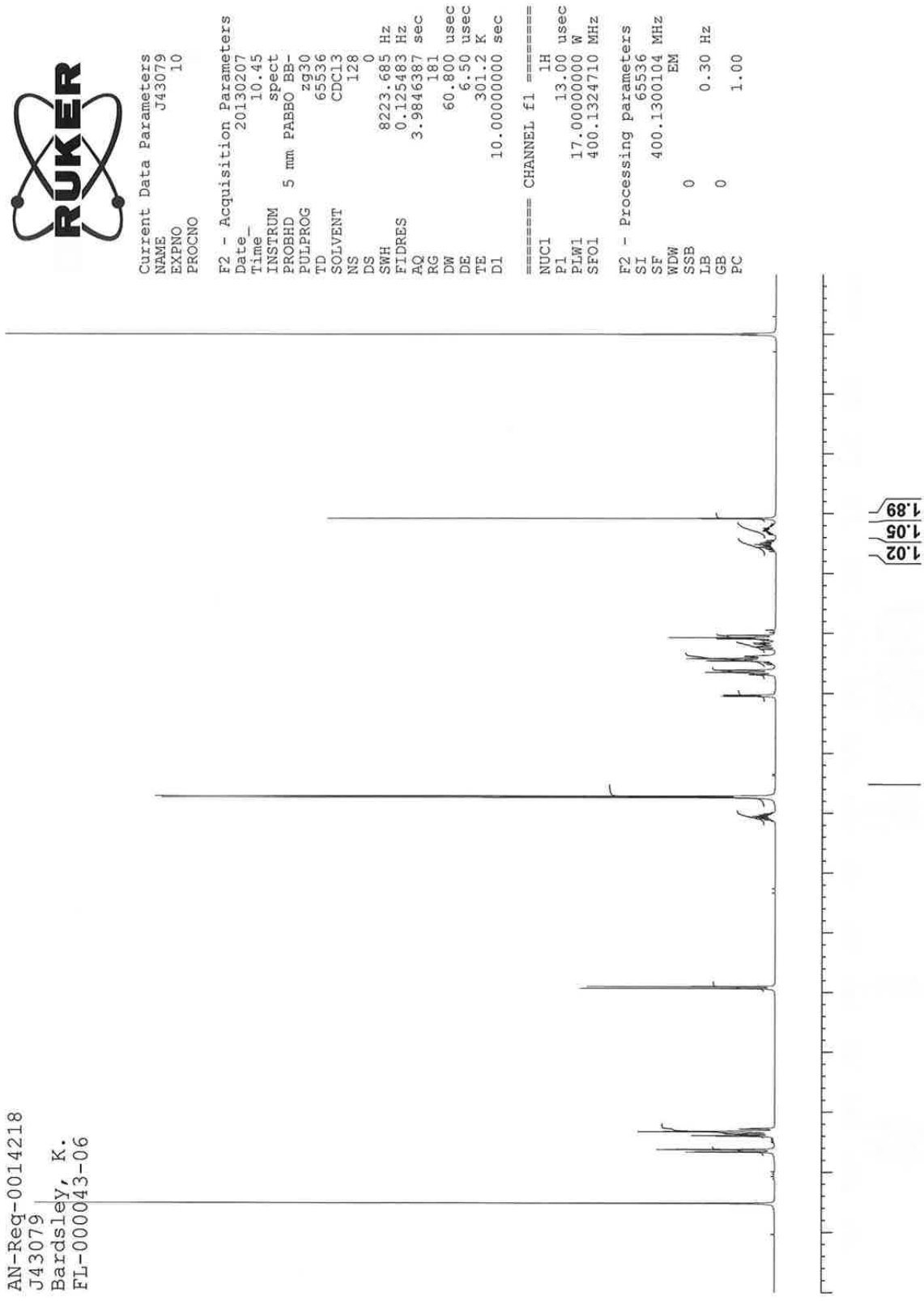
Appendix 35. The LC/MS Chromatograms of F108, F110 and F165



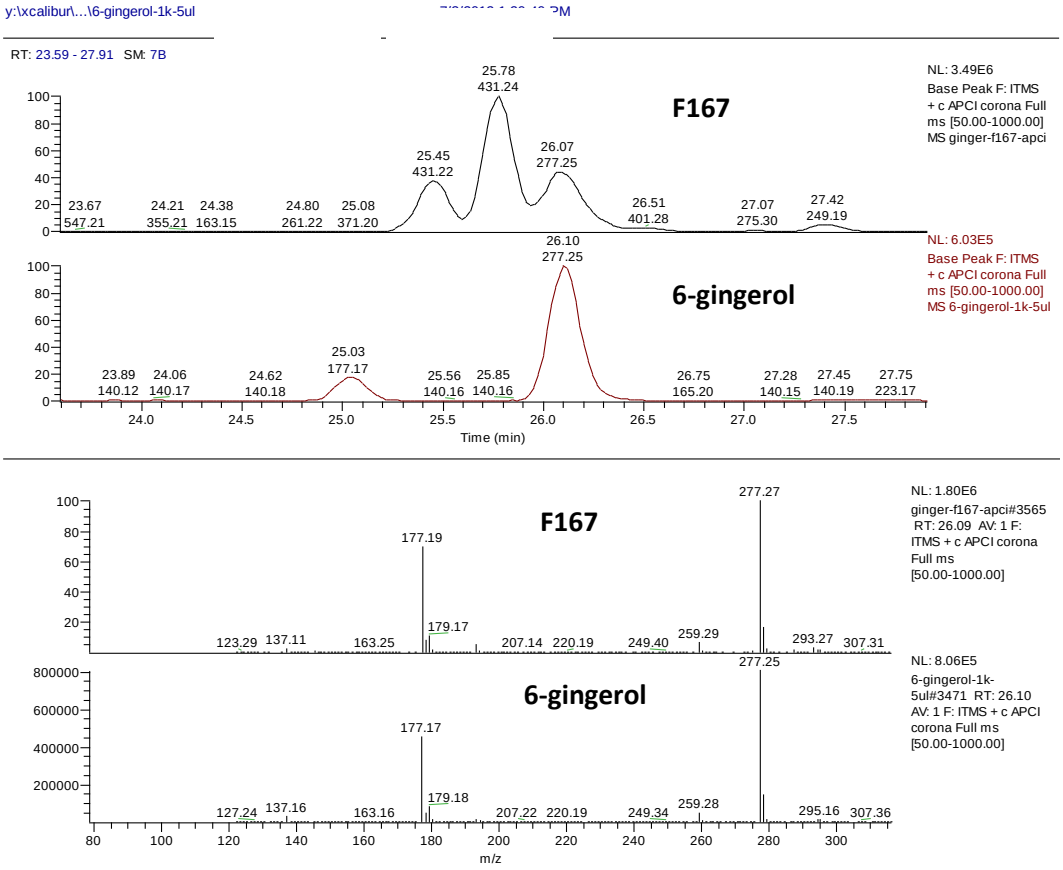
Appendix 36. The LC/MS and MS2 of F126 and the Commercial Standard (Hexahydrocurcumin) for Confirmation



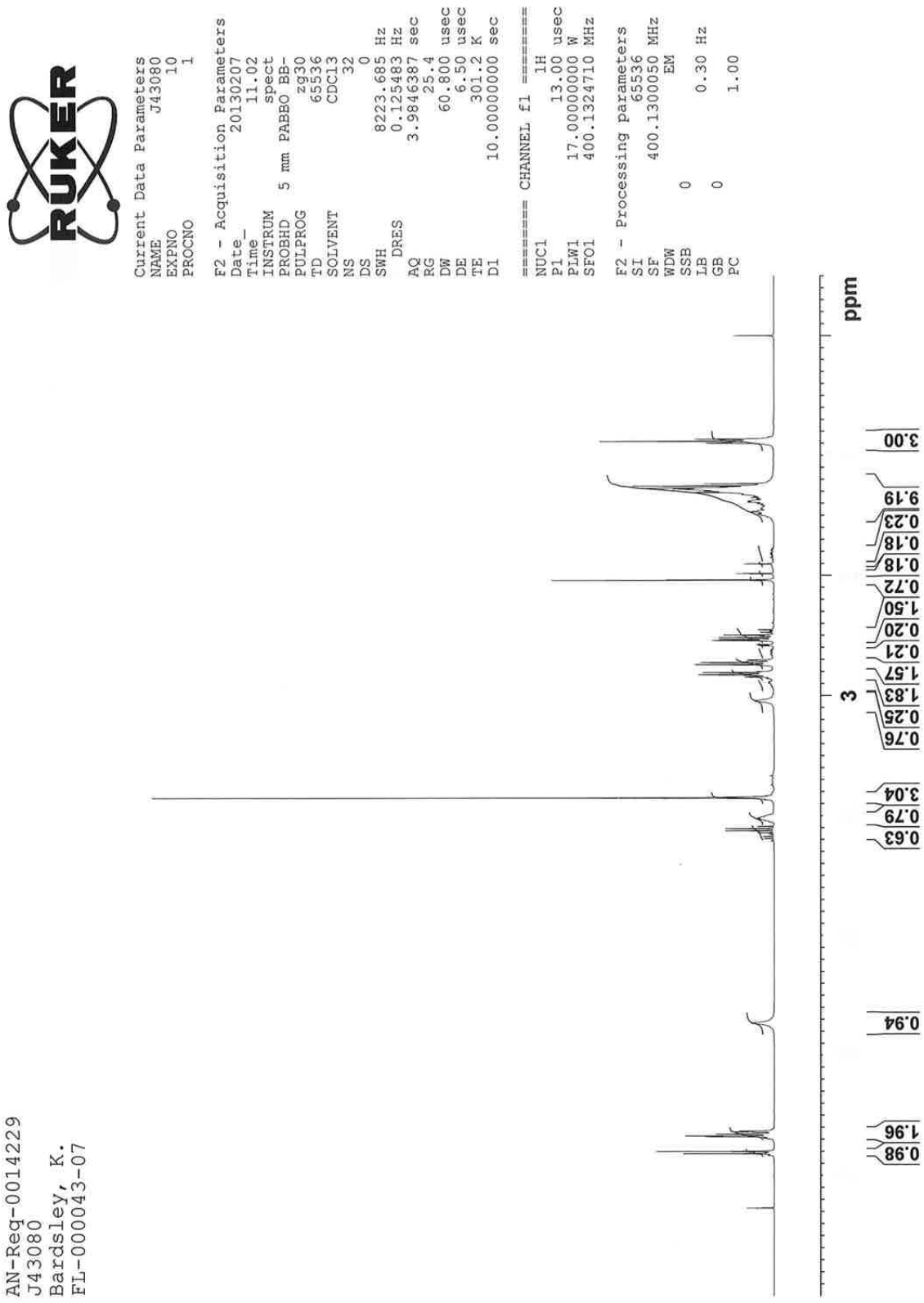
Appendix 37. ^{1}H-NMR of Hexahydrocurcumin



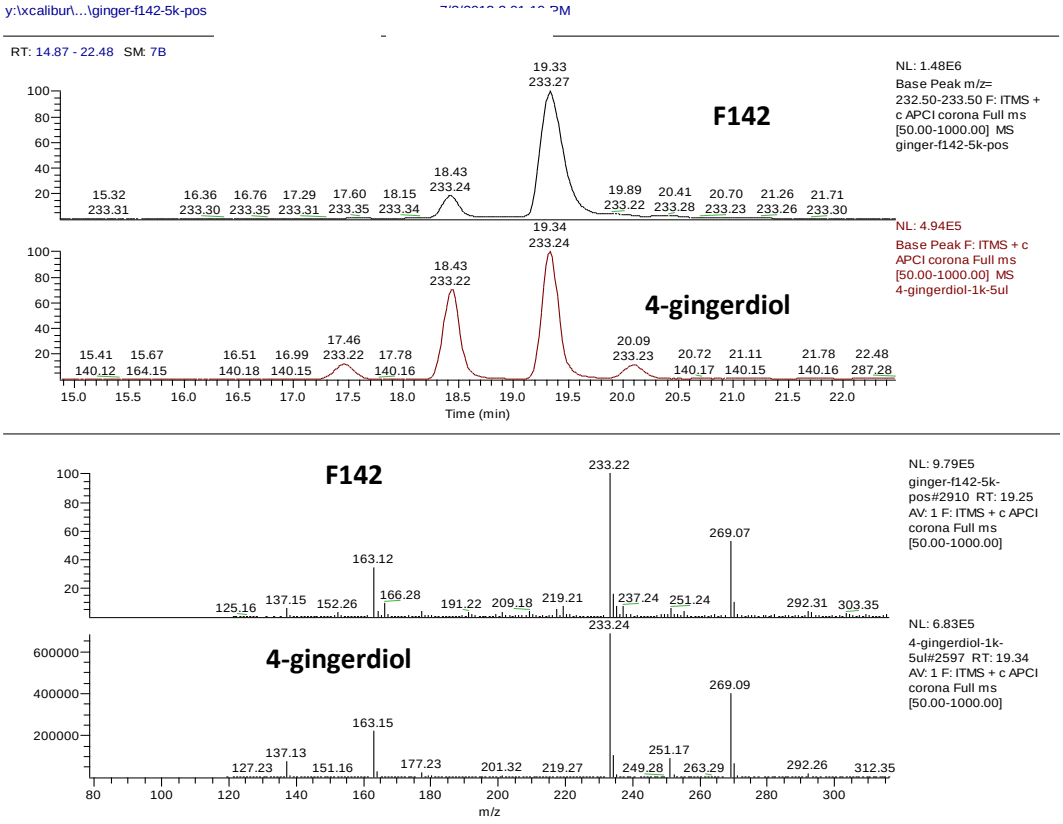
Appendix 38. The LC/MS and MS2 of F167 and the Commercial Standard ([6]-Gingerol) for Confirmation



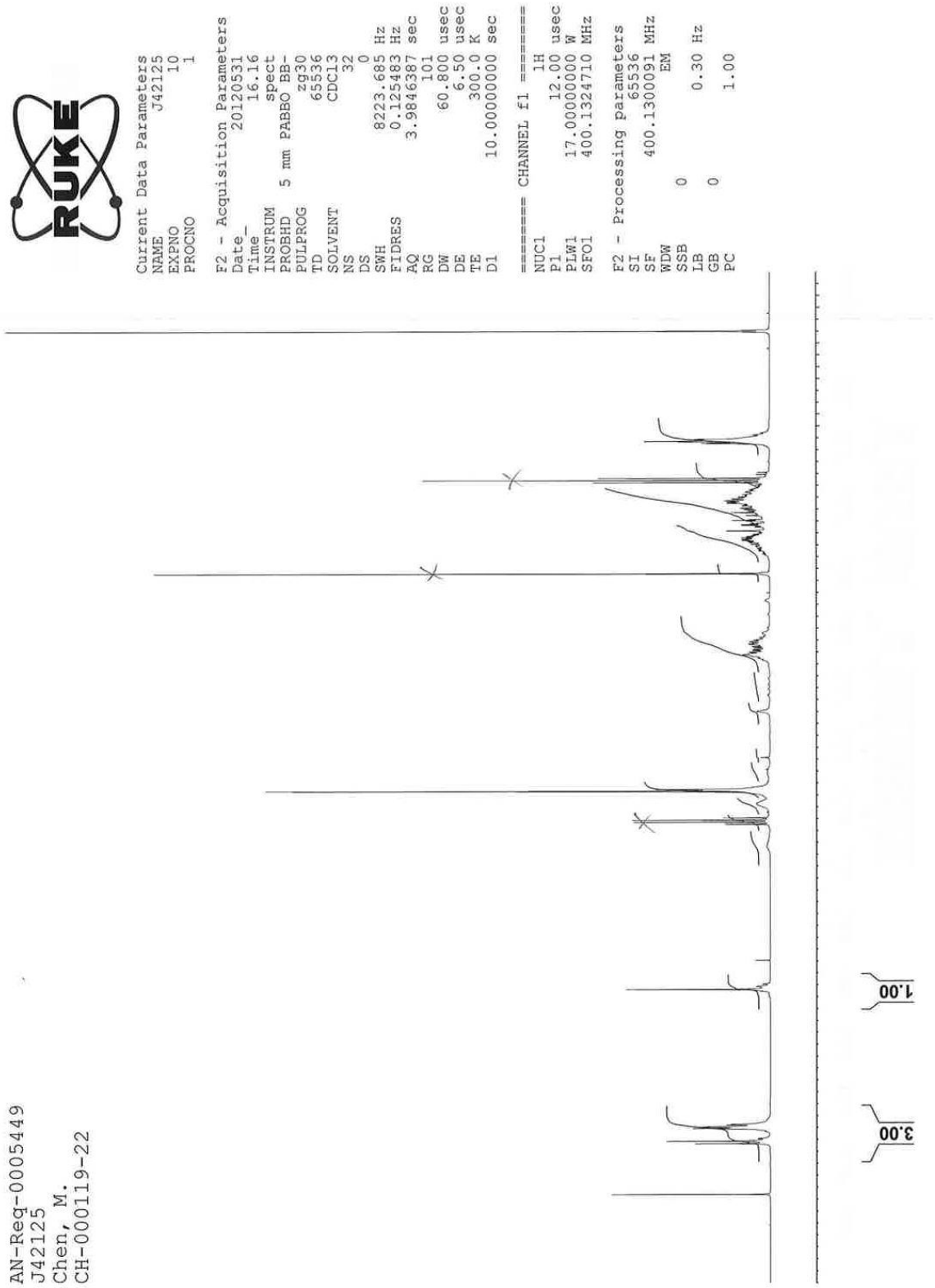
Appendix 39. ^{1}H-NMR of [6]-Gingerol



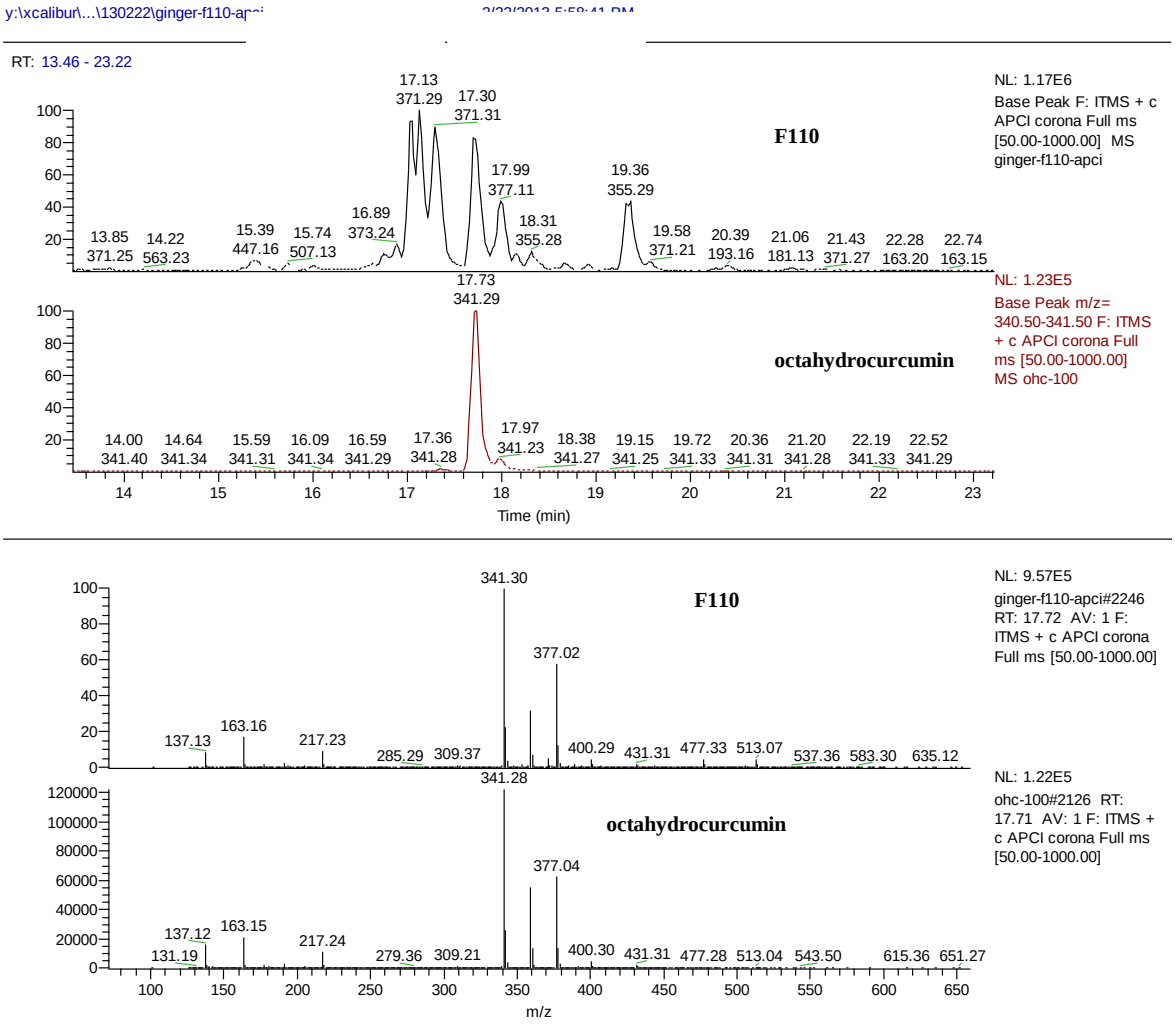
Appendix 40. The LC/MS and MS2 of F142 and the Synthetic Standard ([4]-Gingerdiol) for Confirmation



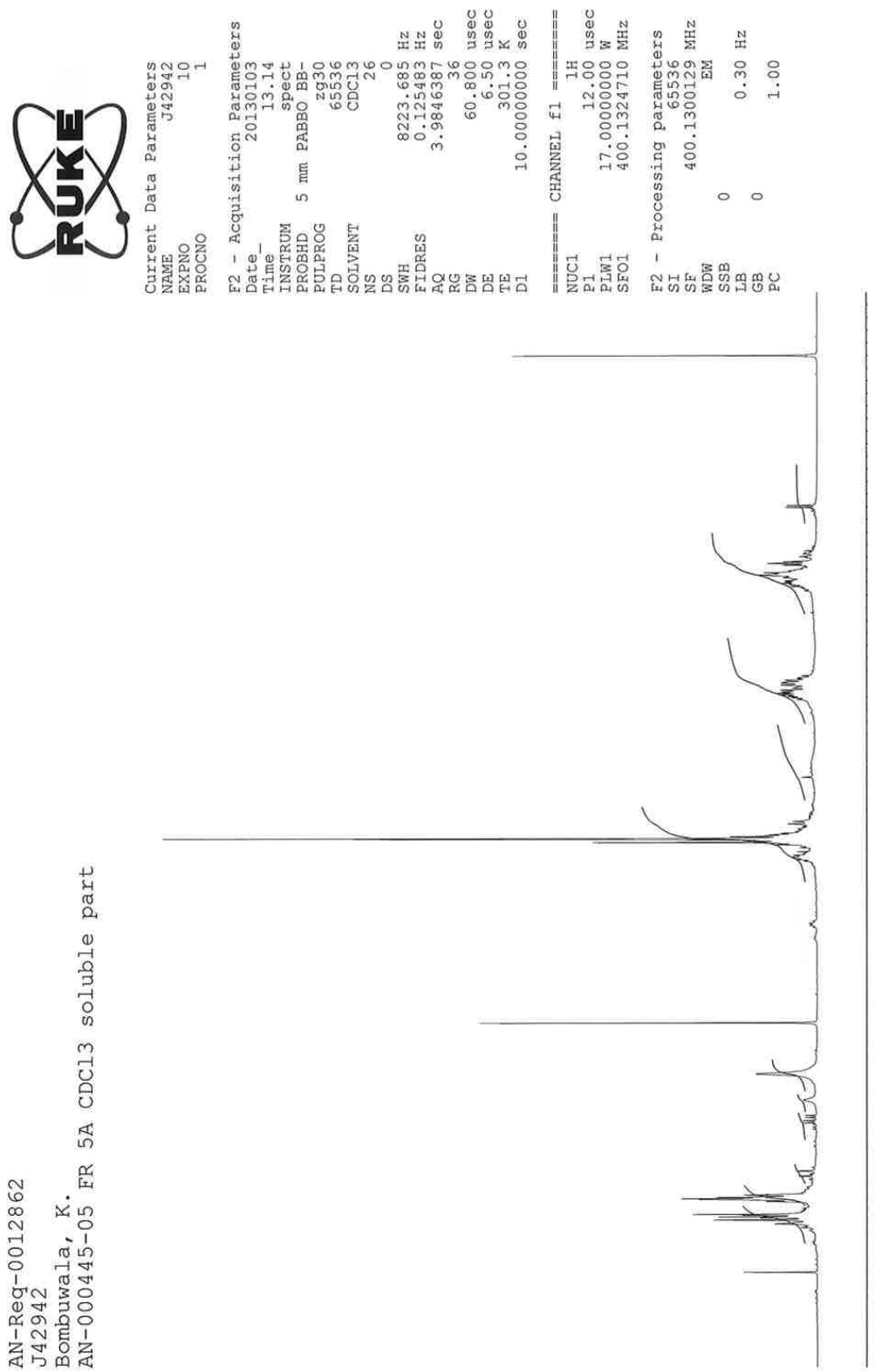
Appendix 41. ^{1}H-NMR of [4]-Gingerdiol



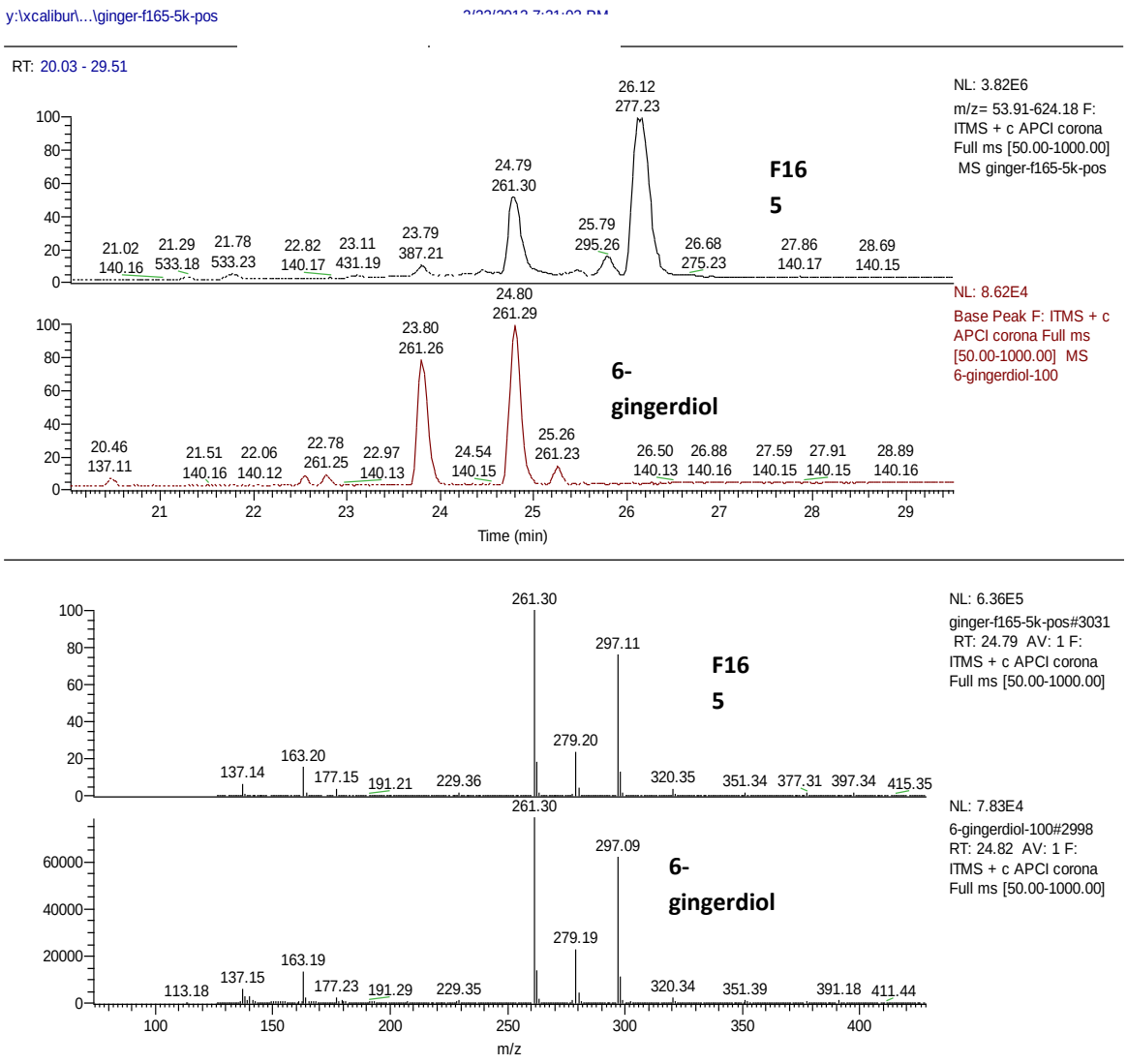
Appendix 42. The LC/MS and MS2 of F110 and the Synthetic Standard (Octahydrocurcumin) for Confirmation



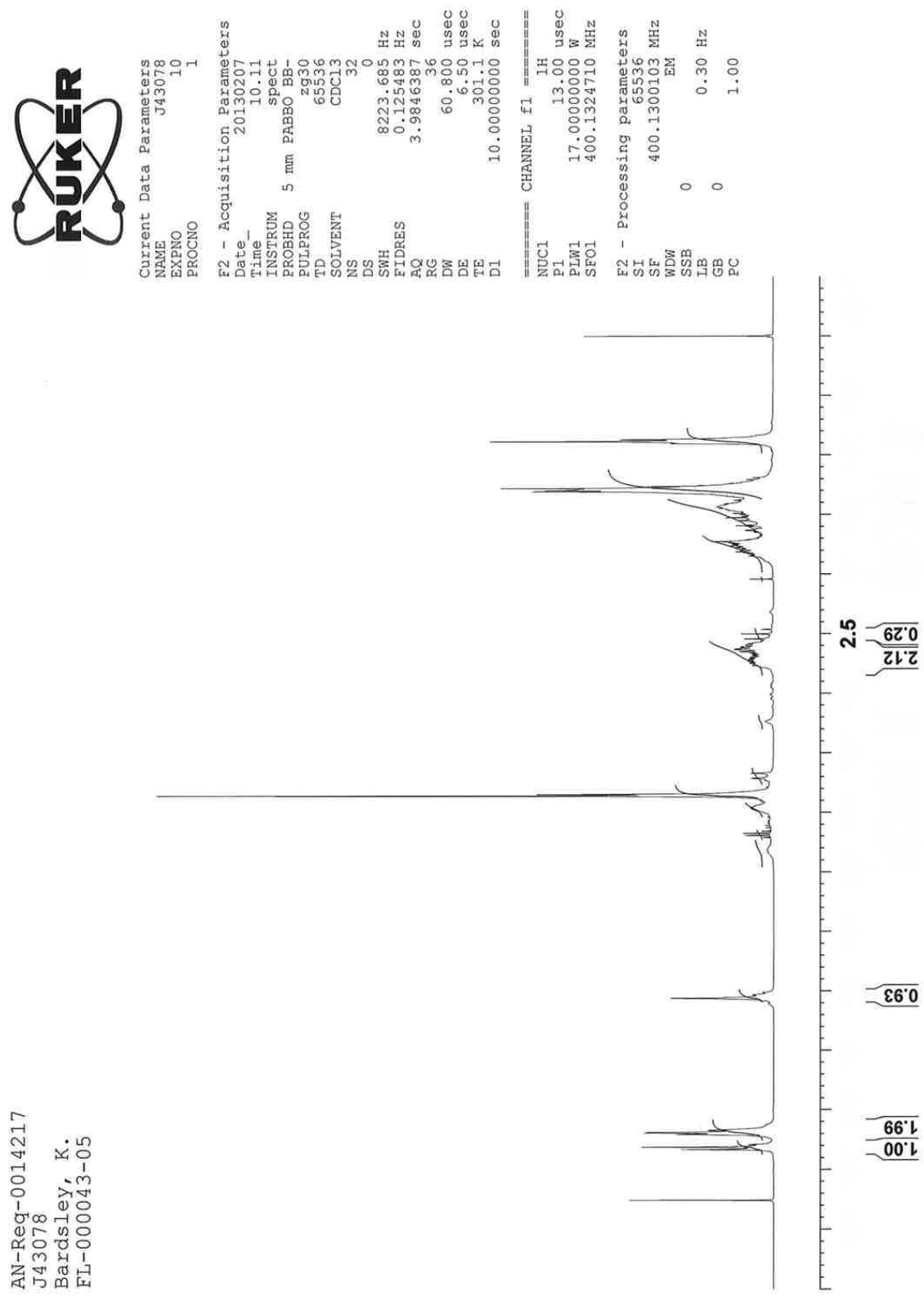
Appendix 43. ^{1}H-NMR of Octahydrocurcumin Pure Fraction *5A*



Appendix 44. The LC/MS and MS2 of F165 and the Synthetic Standard ([6]-Gingerdiol) for Confirmation



Appendix 45. ^{1}H-NMR of [6]-Gingerdiol



Appendix 46. Optimizing Antioxidant Concentrations Using ORAC Assay

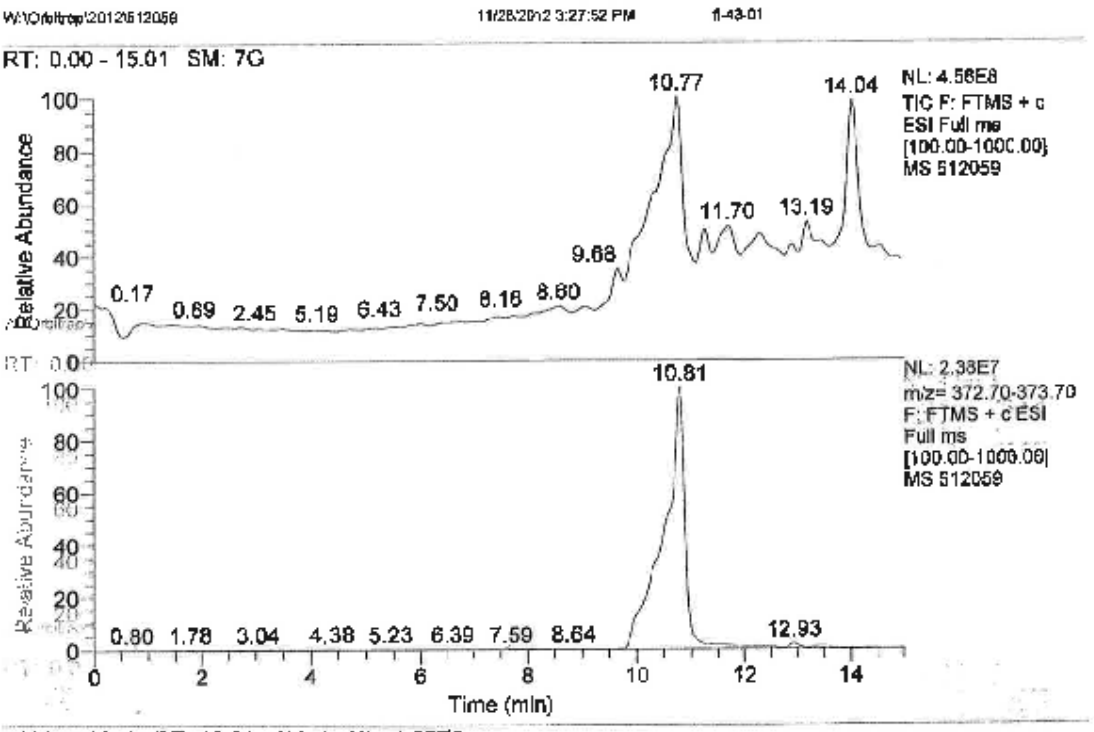
Testing Levels:	4-Gingerol 0.001%	4-Gingerol 0.002%	4-Gingerol 0.004%	4-Gingerol 0.006%	4-Gingerol 0.008%	4-Gingerol 0.01%	4-Gingerol 0.02%	4-Gingerol 0.04%	4-Gingerol 0.06%	4-Gingerol 0.08%	4-Gingerol 0.1%	4-Gingerol 1%
Net AUC:	1.7267	5.83064	14.7643	18.6958	18.7414	19.1499	18.9481	19.1819	18.9113	18.6449	18.9131	23.2986
Testing Levels:	4-Shogaol 0.001%	4-Shogaol 0.002%	4-Shogaol 0.004%	4-Shogaol 0.006%	4-Shogaol 0.008%	4-Shogaol 0.01%	4-Shogaol 0.02%	4-Shogaol 0.04%	4-Shogaol 0.06%	4-Shogaol 0.08%	4-Shogaol 0.1%	4-Shogaol 1%
Net AUC:	2.44959	6.7991	13.9186	17.7593	18.6654	18.6985	18.3476	18.5654	18.3312	18.7418	18.6494	19.8228
Testing Levels:	4-Gingerdiol 0.001%	4-Gingerdiol 0.002%	4-Gingerdiol 0.004%	4-Gingerdiol 0.006%	4-Gingerdiol 0.008%	4-Gingerdiol 0.01%	4-Gingerdiol 0.02%	4-Gingerdiol 0.04%	4-Gingerdiol 0.06%	4-Gingerdiol 0.08%	4-Gingerdiol 0.1%	4-Gingerdiol 1%
Net AUC:	1.83642	8.6049	14.6625	18.9771	18.8056	19.2331	19.0613	18.9735	18.8633	18.8811	18.8554	21.9124

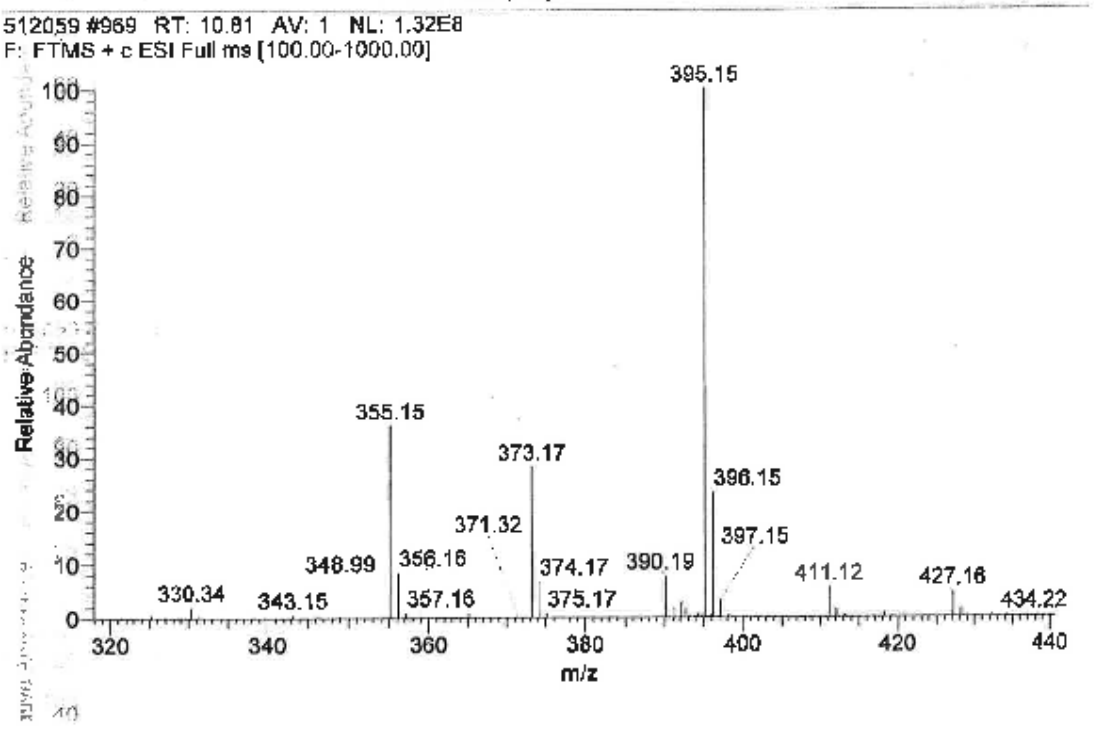
Testing Levels:	6-Gingerol 0.006%	6-Gingerol 0.008%	6-Gingerol 0.01%	6-Gingerol 0.02%	6-Gingerol 0.04%	6-Gingerol 0.06%	6-Gingerol 0.08%	6-Gingerol 0.1%	6-Gingerol 0.2%	6-Gingerol 0.3%	6-Gingerol 1%
Net AUC:	13.7369	18.3366	18.4815	18.4733	18.4099	18.7275	18.851	18.7144	18.5071	18.7502	18.8139
Testing Levels:	S-6-Gingerol 0.008%	S-6-Gingerol 0.02%	S-6-Gingerol 0.1%	S-6-Gingerol 0.6%							
Net AUC:	16.4926	18.5079	23.6127	18.5701							
Testing Levels:	6-Gingerdiol 0.006%	6-Gingerdiol 0.008%	6-Gingerdiol 0.01%	6-Gingerdiol 0.02%	6-Gingerdiol 0.04%	6-Gingerdiol 0.06%	6-Gingerdiol 0.08%	6-Gingerdiol 0.1%	6-Gingerdiol 0.2%	6-Gingerdiol 0.3%	6-Gingerdiol 1%
Net AUC:	17.0711	18.7756	18.6916	18.7365	18.7335	18.8092	18.6673	18.5859	18.5273	18.7873	19.2402

Testing Levels:	THC 0.0001%	THC 0.001%	THC 0.002%	THC 0.004%	THC 0.006%	THC 0.008%	THC 0.01%	THC 0.02%	THC 0.04%	THC 0.06%	THC 0.08%
Net AUC:	-2.5653	3.15036	8.4178	13.0997	15.2232	15.3689	15.2297	15.0316	15.3452	15.0316	15.2133
Testing Levels:	THC 0.1%	THC 0.3%	THC 0.5%	THC 0.8%	THC 1%		HHC 0.0001%	HHC 0.001%	HHC 0.01%	HHC 0.1%	HHC 1%
Net AUC:	15.2247	14.75	14.7461	14.634	16.0468		-3.8236	2.21764	15.3073	15.5896	17.9958
Testing Levels:	OHC 0.0001%	OHC 0.001%	OHC 0.002%	OHC 0.004%	OHC 0.006%	OHC 0.008%	OHC 0.01%	OHC 0.02%	OHC 0.04%	OHC 0.06%	OHC 0.08%
Net AUC:	-1.7363	1.09186	7.2987	12.6366	14.9283	15.2887	15.2639	15.7648	15.4658	15.4144	15.5198
Testing Levels:	OHC 0.1%	OHC 0.3%	OHC 0.5%	OHC 0.8%	OHC 1%						
Net AUC:	15.499	15.964	15.6885	16.3023	17.7887						

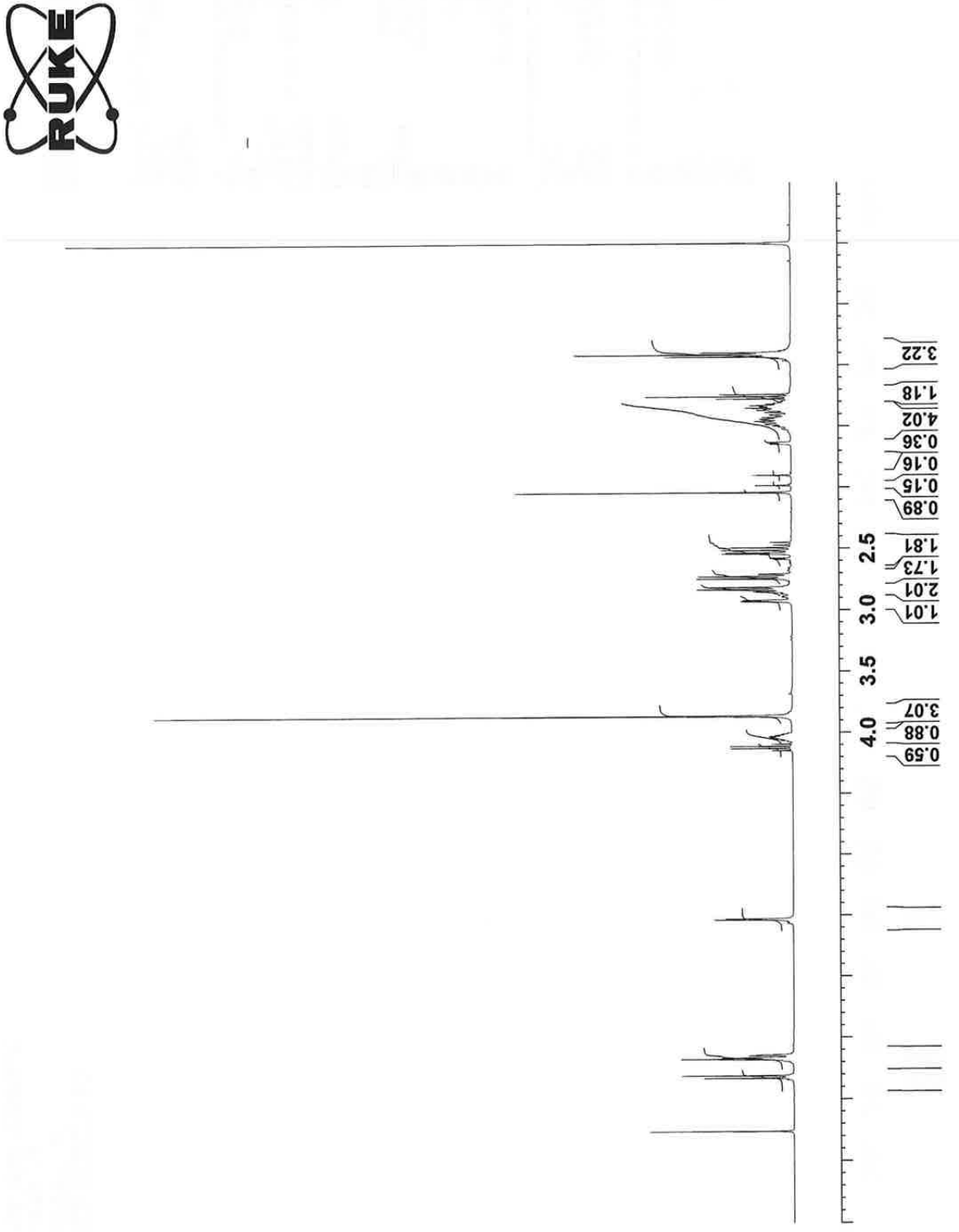
Testing Levels:	Toco-pherols 0.00006%	Toco-pherols 0.0001%	Toco-pherols 0.0005%	Toco-pherols 0.001%	Toco-pherols 0.003%	Toco-pherols 0.005%	Toco-pherols 0.008%	Toco-pherols 0.01%	Toco-pherols 0.03%	Toco-pherols 0.05%	Toco-pherols 0.08%	Toco-pherols 0.1%
Net AUC:	-4.1111	-4.193	-4.4775	-4.441	-4.3605	-3.3452	-4.2552	-3.9621	-3.6099	-3.3168	-2.692	-2.7037

Appendix 47. LCMS of Tetrahydrocurcumin

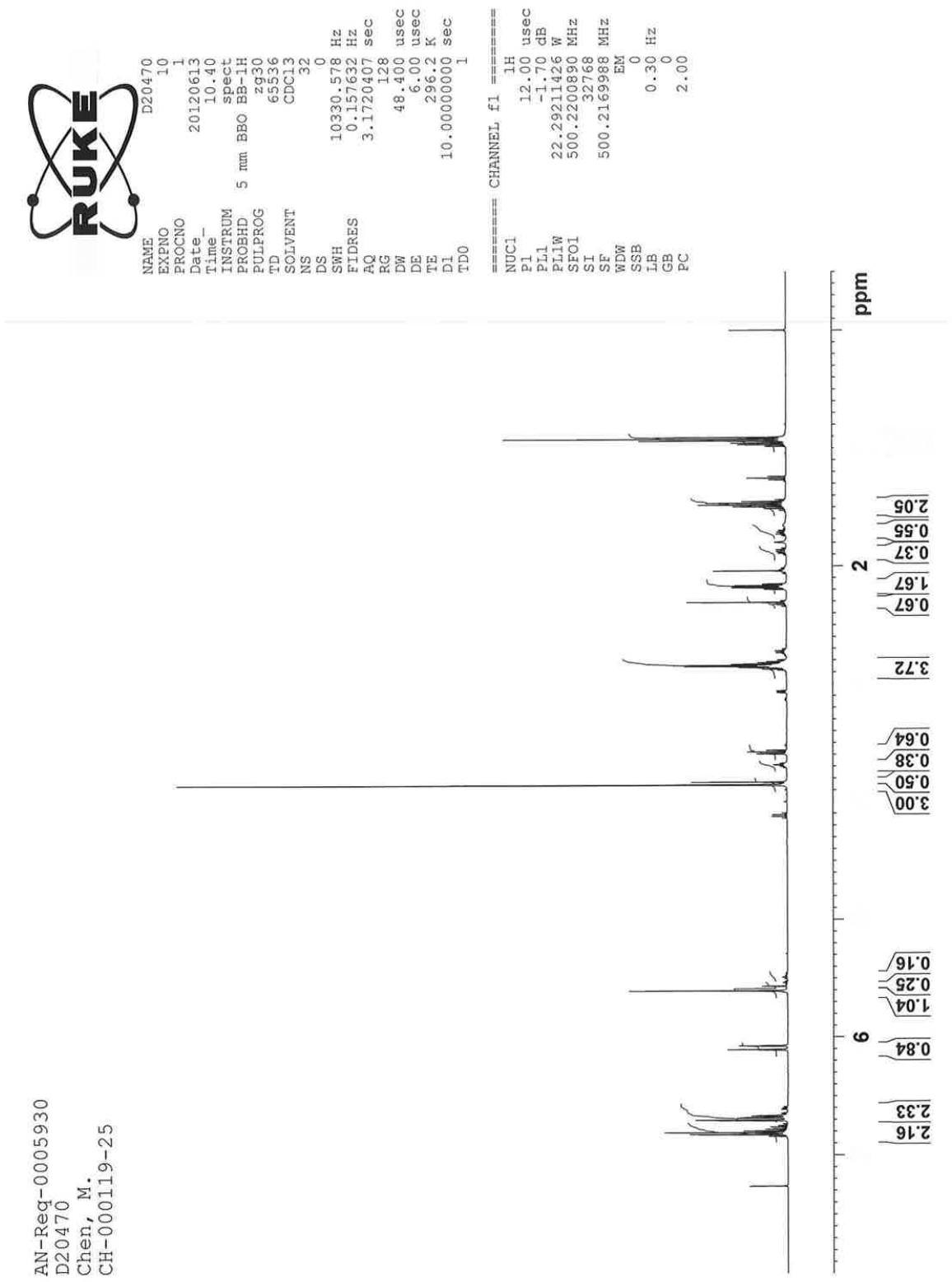




Appendix 48. ^{1}H-NMR of [4]-Gingerol



Appendix 49. ^{1}H-NMR of [4]-Shogaol



Appendix 50. Data Related to First Set of Antioxidants in Lemon Oil

Anti-oxidant	Dilution Used	Amt. of Dilution (g)	Filled to (g)	Target Conc.	Actual Conc. (ppm)	Amt. of Oil (g) for PV	Titrant Volume (mL)	Peroxide Value	Aver-age P.V.	Antioxid ant Conc. in Bvg
4-gingerol, A	0.1% in EtOH	0.108	7.996	12.5 ppm	13.51	5.02	10.55	20.32	21.84	
4-gingerol, B	0.1% in EtOH	0.099	7.996	12.5 ppm	12.38	5.00	12.57	24.44		
4-gingerol, C	0.1% in EtOH	0.102	7.997	12.5 ppm	12.75	5.01	10.75	20.76		1.25ppb
4-gingerdiol, A	0.1% in EtOH	0.1	7.994	12.5 ppm	12.51	5.02	12.35	23.90	22.87	
4-gingerdiol, B	0.1% in EtOH	0.1	7.998	12.5 ppm	12.50	5.01	11.75	22.75		1.25ppb
4-gingerdiol, C	0.1% in EtOH	0.106	7.998	12.5 ppm	13.25	5.04	11.42	21.96		
4-shogaol, A	0.1% in EtOH	0.108	7.996	12.5 ppm	13.51	5.03	10.82	20.82	21.72	
4-shogaol, B	0.1% in EtOH	0.1	8.087	12.5 ppm	12.37	5.01	11.62	22.50		
4-shogaol, C	0.1% in EtOH	0.107	8.002	12.5 ppm	13.37	5.01	11.29	21.84		1.25ppb
6-gingerol, A	1% in EtOH	0.104	8.02	125 ppm	129.68	5.01	12.81	24.87	23.04	
6-gingerol, B	1% in EtOH	0.101	8.008	125 ppm	126.12	5.00	11.56	22.42		12.5ppb
6-gingerol, C	1% in EtOH	0.108	7.997	125 ppm	135.05	5.00	11.26	21.82		
~~S-6-gingerol, A~~	~~0.1% in Acetone~~	~~1.002~~	~~8.177~~	~~125 ppm~~	~~122.54~~	~~5.18~~	~~17.64~~	~~33.38~~	~~34.79~~	
~~S-6-gingerol, B~~	~~0.1% in Acetone~~	~~0.989~~	~~8.304~~	~~125 ppm~~	~~119.10~~	~~5.00~~	~~19.42~~	~~38.14~~		
~~S-6-gingerol, C~~	~~0.1% in Acetone~~	~~1.007~~	~~8.129~~	~~125 ppm~~	~~123.88~~	~~5.01~~	~~16.81~~	~~32.85~~		
6-gingerdiol, A	1% in EtOH	0.064	8.03	75 ppm	79.70	5.01	11.90	23.05	21.40	12.5ppb
6-gingerdiol, B	1% in EtOH	0.06	8.008	75 ppm	74.93	5.00	12.00	23.30		

6-gingerdiol, C	1% in EtOH	0.065	7.995	75 ppm	81.30	5.00	9.28	17.86		
6-shogaol, A	1% in EtOH	0.099	8.017	125 ppm	123.49	5.00	13.01	25.32	25.65	12.5ppb
6-shogaol, B	1% in EtOH	0.101	8.008	125 ppm	126.12	5.07	14.64	28.19		
6-shogaol, C	1% in EtOH	0.115	8.02	125 ppm	143.39	5.01	12.10	23.45		
8-gingerol, A	1% in EtOH	0.1	8.002	125 ppm	124.97	5.01	14.98	29.20	25.32	
8-gingerol, B	1% in EtOH	0.097	8.021	125 ppm	120.93	5.07	12.44	23.85		12.5ppb
8-gingerol, C	1% in EtOH	0.101	8.015	125 ppm	126.01	5.01	11.83	22.91		
8-shogaol, A	1% in EtOH	0.099	8.004	125 ppm	123.69	5.03	10.82	20.82	21.03	12.5ppb
8-shogaol, B	1% in EtOH	0.099	8.01	125 ppm	123.60	5.02	10.35	19.92		
8-shogaol, C	1% in EtOH	0.104	8.009	125 ppm	129.85	5.02	11.57	22.35		
10-gingerol, A	1% in EtOH	0.099	8.063	125 ppm	122.78	5.01	11.88	23.01	24.18	
10-gingerol, B	1% in EtOH	0.105	7.996	125 ppm	131.32	5.01	13.12	25.49		
10-gingerol, C	1% in EtOH	0.098	8.006	125 ppm	122.41	5.03	12.44	24.04		12.5ppb
10-shogaol, A	1% in EtOH	0.102	8.003	125 ppm	127.45	5.08	15.12	29.07	26.69	
10-shogaol, B	1% in EtOH	0.1	7.998	125 ppm	125.03	5.07	13.60	26.13		12.5ppb
10-shogaol, C	1% in EtOH	0.103	7.992	125 ppm	128.88	5.02	12.83	24.86		
Tetrahydrocurcumin, A	1% in EtOH	0.044	7.992	50 ppm	55.06	5.00	9.13	17.56	18.68	
Tetrahydrocurcumin, B	1% in EtOH	0.041	8	50 ppm	51.25	5.01	9.29	17.84		5ppb
Tetrahydrocurcumin, C	1% in EtOH	0.046	8.006	50 ppm	57.46	5.14	10.95	20.62		

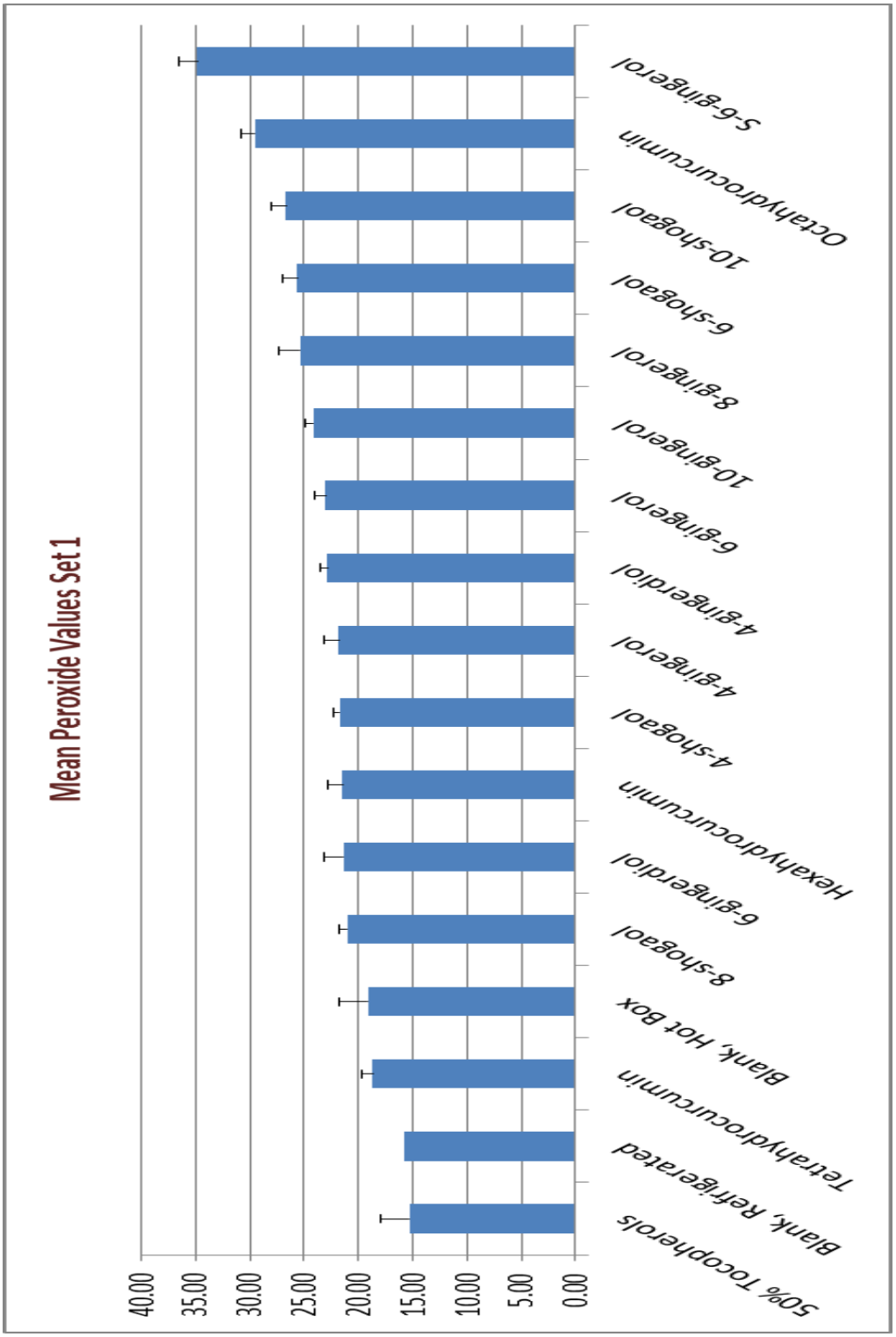
Hexahydrocurcumin, A	1% in Acetone	0.102	8.005	125 ppm	127.42	5.02	12.51	24.22	21.49	
Hexahydrocurcumin, B	1% in Acetone	0.1	8.002	125 ppm	124.97	5.00	10.42	20.14		12.5ppb
Hexahydrocurcumin, C	1% in Acetone	0.101	8.009	125 ppm	126.11	5.01	10.42	20.10		
Octahydrocurcumin, A	0.1% in EtOH	0.214	8.004	25 ppm	26.74	5.08	15.03	28.90	29.49	2.5ppb
Octahydrocurcumin, B	0.1% in EtOH	0.204	7.994	25 ppm	25.52	5.04	14.25	27.58		
Octahydrocurcumin, C	0.1% in EtOH	0.203	8.02	25 ppm	25.31	5.03	16.44	31.99		
50% Tocopherols, A	10% in EtOH	0.04	8.044	500 ppm	497.27	5.00	6.81	12.92	15.26	25ppb
50% Tocopherols, B	10% in EtOH	0.048	8.105	500 ppm	592.23	5.00	10.62	20.54		
50% Tocopherols, C	10% in EtOH	0.041	8.015	500 ppm	511.54	5.02	6.53	12.31		
~~Blank, Refrigerated, A~~			~~8.07~~			~~5.03~~	~~7.07~~	~~13.35~~	~~Old Pipette~~	
~~Blank, Refrigerated, B~~			~~8.348~~			~~5.00~~	~~7.46~~	~~14.21~~	~~Old Pipette~~	
~~Blank, Refrigerated, C~~			~~8.084~~			~~5.04~~	~~7.12~~	~~13.43~~	~~Old Pipette~~	
Blank, Refrigerated, D						5.10	8.44	15.86	15.86	0
~~Blank, Hot Box, A~~			~~8.136~~			~~5.00~~	~~**6.50**~~	~~**12.30**~~	~~Old Pipette~~	
Blank, Hot Box, B			8.004			5.00	11.19	21.68	19.10	0
Blank, Hot Box, C			8.089			5.02	8.64	16.51		

*Note$_1$: Only those in bold were tasted. Note$_2$: *S*-6-gingerol (with strikethrough) was not included in tastings because of elevated levels of ethanol. All other strikethroughs reflect the use of a broken pipette, therefore, the results were discarded.

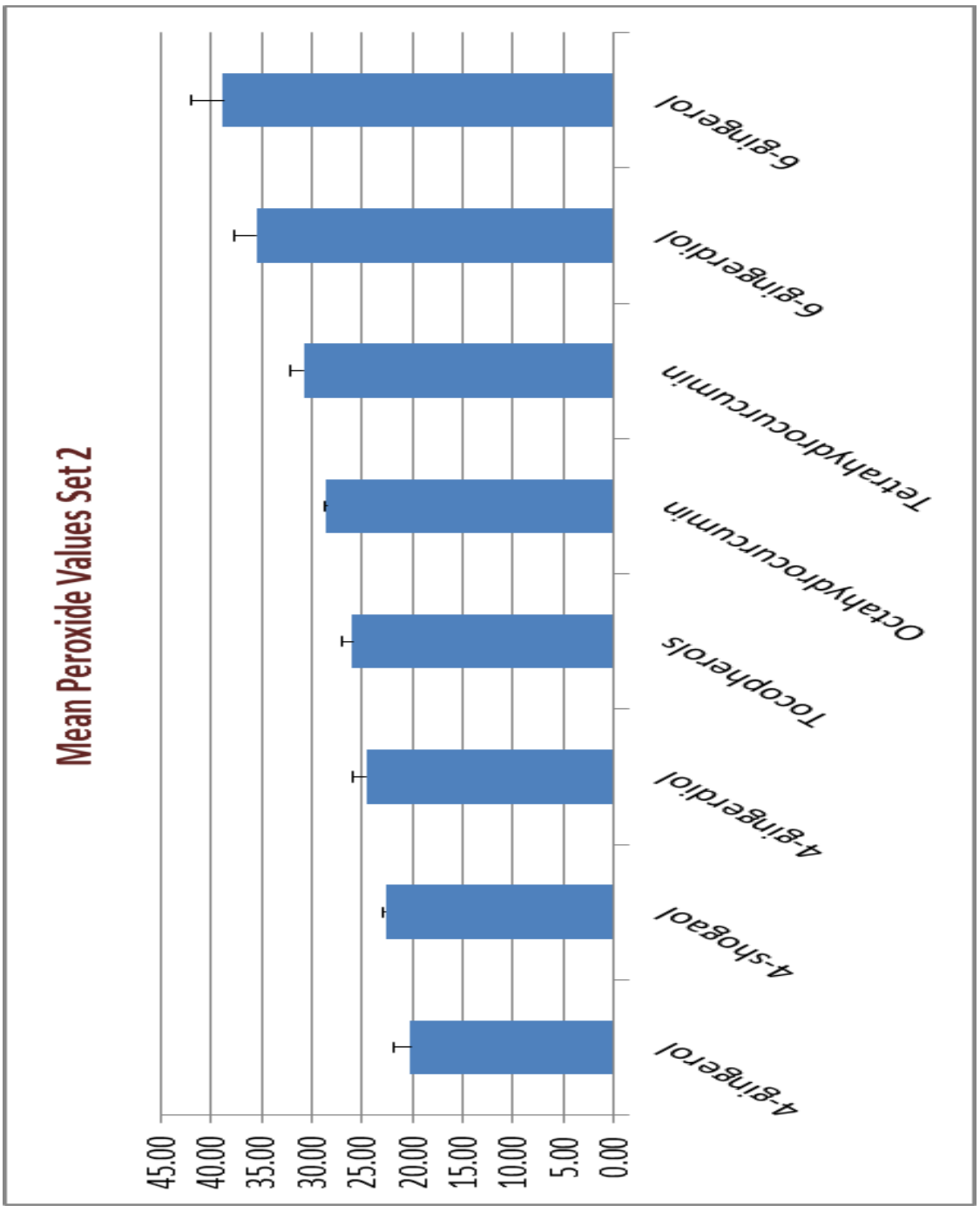
Appendix 51. Data Related to Second Set of Antioxidants in Lemon Oil

Anti-oxidant	Dilution Used	Amt. of Dilution (g)	Filled to (g)	Target Conc.	Actual Conc. (ppm)	Amt. of Oil (g) for PV	Titrant Volume (mL)	Peroxide Value	Aver-age P.V.
4-gingerol, A	1% in EtOH	0.078	7.995	0.01%	97.56	5.00	9.20	18.00	20.20
4-gingerol, B	1% in EtOH	0.079	8.064	0.01%	97.97	5.01	11.90	23.35	
4-gingerol, C	1% in EtOH	0.083	8.005	0.01%	103.69	4.99	9.80	19.24	
4-gingerdiol, A	1% in EtOH	0.077	7.999	0.01%	96.26	5.00	11.60	22.80	24.59
4-gingerdiol, B	1% in EtOH	0.081	8.02	0.01%	101.00	5.00	12.20	24.00	
4-gingerdiol, C	1% in EtOH	0.088	8.111	0.01%	108.49	5.00	13.69	26.98	
4-shogaol, A	1% in EtOH	0.08	8.001	0.01%	99.99	4.99	11.73	23.11	22.66
4-shogaol, B	1% in EtOH	0.081	7.989	0.01%	101.39	5.00	11.27	22.14	
4-shogaol, C	1% in EtOH	0.082	8.092	0.01%	101.33	5.00	11.56	22.72	
6-gingerol, A	1% in EtOH	0.798	8.066	0.10%	989.34	5.01	20.19	39.90	38.78
6-gingerol, B	1% in EtOH	0.829	8.214	0.10%	1009.25	5.01	22.17	43.85	
6-gingerol, C	1% in EtOH	0.853	8.12	0.10%	1050.49	5.11	16.85	32.58	
6-gingerdiol, A	1% in EtOH	0.48	8.008	0.06%	599.40	5.00	20.00	39.60	35.48
6-gingerdiol, B	1% in EtOH	0.48	8.05	0.06%	596.27	5.00	17.42	34.44	
6-gingerdiol, C	1% in EtOH	0.477	8.151	0.06%	585.20	4.99	16.37	32.40	
Tetrahydro curcumin, A	1% in EtOH	0.317	7.997	0.04%	396.40	5.01	17.03	33.59	30.80
Tetrahydro curcumin, B	1% in EtOH	0.326	8.026	0.04%	406.18	5.01	14.84	29.22	
Tetrahydro curcumin, C	1% in EtOH	0.322	7.997	0.04%	402.65	5.00	15.00	29.60	
Octahydrocurcumin, A	1% in EtOH	0.158	7.996	0.02%	197.60	5.01	11.15	~~21.86~~	
Octahydrocurcumin, B	1% in EtOH	0.16	8.008	0.02%	199.80	5.00	14.52	28.64	28.53
Octahydrocurcumin, C	1% in EtOH	0.166	8.151	0.02%	203.66	5.06	14.58	28.42	
Tocopherols, A	10% in EtOH	0.158	7.995	0.10%	1976.24	5.00	13.47	26.54	25.96
Tocopherols, B	10% in EtOH	0.161	8	0.10%	2012.50	4.99	12.18	24.01	
Tocopherols, C	10% in EtOH	0.16	7.991	0.10%	2002.25	5.01	13.89	27.33	

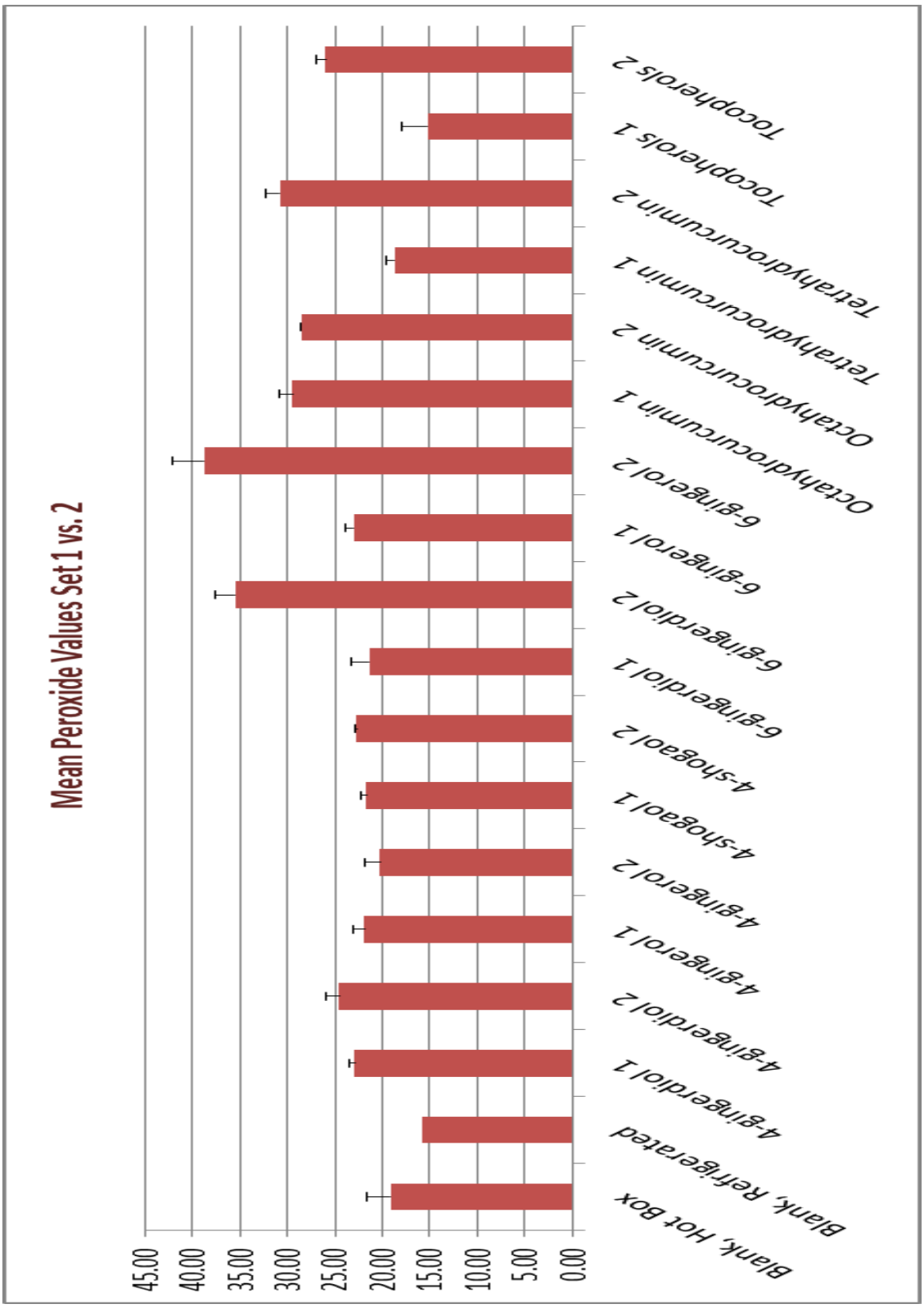
Appendix 52. Bar Chart to Display Significance of First Set of Lemon Oils



Appendix 53. Bar Chart to Display Significance of Second Set of Lemon Oils



Appendix 54. Bar Chart to Display Significance of Paired Comparisons



Appendix 55. Peak Areas from GC of Degradation Markers for Each Lemon Oil

Argentinian Lemon 1X	*p*-Cymene	Limonene	Limonene oxide	α-Terpineol	Geranial	Neral	Carvone
Original Starting Oil	0.19	66.34	0	0.02	2.02	1.17	0
Control Fridge A	0.32	66.47	0.01	0.02	1.97	1.13	0.03
Control Fridge B	0.32	66.48	0.01	0.02	1.97	1.13	0.03
Control Fridge C	0.32	66.51	0.01	0.02	1.94	1.11	0.03
Control Fridge D	**0.28**	**66.52**	**0.01**	**0.02**	**1.96**	**1.13**	**0.03**
Control Hot Box A	0.67	66.43	0.06	0.02	1.96	1.12	0.03
Control Hot Box B	**0.81**	**66.43**	**0.06**	**0.02**	**1.94**	**1.11**	**0.03**
Control Hot Box C	0.7	66.45	0.07	0.02	1.92	1.1	0.03
4-Gingerol A	0.61	66.1	0.04	0.02	1.91	1.11	0.03
4-Gingerol B	0.68	66.16	0.06	0.02	1.88	1.09	0.03
4-Gingerol C	**0.57**	**66.19**	**0.04**	**0.02**	**1.88**	**1.1**	**0.02**
4-Gingerdiol A	0.62	66.15	0.04	0.02	1.94	1.12	0.03
4-Gingerdiol B	**0.55**	**66.17**	**0.04**	**0.02**	**1.9**	**1.11**	**0.02**
4-Gingerdiol C	0.66	66.13	0.05	0.02	1.94	1.12	0.03
4-Shogaol A	0.64	66.18	0.04	0.02	1.85	1.08	0.03
4-Shogaol B	0.62	66.2	0.04	0.02	1.87	1.09	0.03
4-Shogaol C	**0.68**	**66.22**	**0.05**	**0.02**	**1.86**	**1.09**	**0.03**
6-Gingerol A	0.62	66.17	0.04	0.02	1.92	1.12	0.03
6-Gingerol B	**0.53**	**66.15**	**0.04**	**0.02**	**1.92**	**1.12**	**0.02**
6-Gingerol C	0.59	66.17	0.04	0.02	1.92	1.12	0.03
6-Gingerdiol A	**0.7**	**66.23**	**0.06**	**0.02**	**1.97**	**1.14**	**0.03**
6-Gingerdiol B	0.62	66.25	0.04	0.02	1.93	1.11	0.03
6-Gingerdiol C	0.65	66.27	0.04	0.02	1.95	1.12	0.03
6-Shogaol A	**0.73**	**66.13**	**0.06**	**0.02**	**1.96**	**1.13**	**0.03**
6-Shogaol B	0.75	66.14	0.06	0.02	1.86	1.09	0.03
6-Shogaol C	0.64	66.17	0.06	0.02	1.84	1.08	0.03
8-Gingerol A	0.66	66.16	0.06	0.02	1.88	1.1	0.03
8-Gingerol B	**0.73**	**66.19**	**0.06**	**0.02**	**1.89**	**1.1**	**0.02**
8-Gingerol C	0.66	66.17	0.06	0.02	1.87	1.09	0.02
8-Shogaol A	**0.65**	**66.17**	**0.06**	**0.02**	**1.87**	**1.09**	**0.03**
8-Shogaol B	0.58	66.17	0.04	0.02	1.87	1.09	0.02
8-Shogaol C	0.58	66.21	0.04	0.02	1.87	1.09	0.02
10-Gingerol A	0.62	66.16	0.04	0.02	1.9	1.1	0.02
10-Gingerol B	0.77	66.21	0.06	0.02	1.92	1.12	0.03
10-Gingerol C	**0.76**	**66.18**	**0.06**	**0.02**	**1.87**	**1.09**	**0.03**
10-Shogaol A	0.65	66.14	0.06	0.02	1.76	1.03	0.02
10-Shogaol B	**0.63**	**66.22**	**0.04**	**0.02**	**1.76**	**1.03**	**0.02**
10-Shogaol C	0.68	66.27	0.06	0.02	1.7	1	0.02

Tetrahydrocurcumin A	0.78	66.33	0.06	0.02	1.85	1.07	0.03
Tetrahydrocurcumin B	**0.73**	**66.34**	**0.06**	**0.02**	**1.86**	**1.08**	**0.03**
Tetrahydrocurcumin C	0.77	66.31	0.06	0.02	1.84	1.07	0.03
Hexahydrocurcumin A	0.97	66.34	0.07	0.02	1.86	1.07	0.03
Hexahydrocurcumin B	**0.76**	**66.53**	**0.06**	**0.02**	**1.79**	**1.04**	**0.03**
Hexahydrocurcumin C	0.93	66.52	0.07	0.02	1.82	1.05	0.04
Octahydrocurcumin A	**0.64**	**65.75**	**0.06**	**0.02**	**1.79**	**1.05**	**0.03**
Octahydrocurcumin B	0.75	65.77	0.06	0.02	1.78	1.05	0.04
Octahydrocurcumin C	0.69	65.93	0.06	0.02	1.75	1.04	0.03
Tocopherols A	0.58	66.46	0.06	0.02	1.92	1.01	0.03
Tocopherols B	0.66	66.5	0.07	0.02	1.91	0.97	0.02
Tocopherols C	0.59	67.17	0	0	1.82	0.93	0
2: 4-Gingerol A	0.58	66.19	0.04	0.02	1.81	1.06	0.02
2: 4-Gingerol B	0.72	66.22	0.06	0.02	1.83	1.07	0.03
2: 4-Gingerol C	0.61	66.28	0.04	0.02	1.68	0.99	0.02
2: 4-Gingerdiol A	0.71	66.33	0.06	0.02	1.79	1.05	0.02
2: 4-Gingerdiol B	0.72	66.37	0.06	0.02	1.87	1.09	0.02
2: 4-Gingerdiol C	0.68	66.33	0.06	0.02	1.79	1.05	0.02
2: 4-Shogaol A	0.61	66.31	0.05	0.02	1.79	1.05	0.02
2: 4-Shogaol B	0.69	66.31	0.06	0.02	1.84	1.08	0.02
2: 4-Shogaol C	0.66	66.3	0.06	0.02	1.74	1.03	0.02
2: 6-Gingerol A	0.59	63.86	0.04	0.02	1.66	0.98	0.02
2: 6-Gingerol B	0.62	63.62	0.04	0.02	1.67	0.99	0.02
2: 6-Gingerol C	0.51	63.64	0.03	0.02	1.76	1.03	0.02
2: 6-Gingerdiol A	0.61	64.99	0.04	0.02	1.72	1.02	0.02
2: 6-Gingerdiol B	0.56	65.06	0.04	0.02	1.71	1.01	0.02
2: 6-Gingerdiol C	0.56	65.05	0.04	0.02	1.68	1	0.02
2: Tetra-hydrocurcumin A	0.64	65.39	0.04	0.02	1.73	1.02	0.02
2: Tetra-hydrocurcumin B	0.52	65.48	0.04	0.02	1.66	0.99	0.01
2: Tetra-hydrocurcumin C	0.55	65.42	0.04	0.02	1.76	1.04	0.02
2: Octa-hydrocurcumin A	0.54	66.04	0.04	0.02	1.71	1.02	0.01
2: Octahydrocurcumin B	0.53	66.09	0.04	0.02	1.72	1.02	0.01
2: Octahydrocurcumin C	0.54	65.94	0.05	0.02	1.77	1.05	0.02
2: Tocopherols A	0.52	66.04	0.04	0.02	2	1.08	0.03
2: Tocopherols B	0.47	65.97	0.04	0.02	1.81	1	0.02
2: Tocopherols C	0.5	65.99	0.04	0.02	1.76	0.97	0.02

Appendix 56. GC of Original Starting Argentinian Lemon Oil