COMPARISON AND INTEGRATION OF ANALYTICAL METHODS FOR THE CHARACTERIZATION OF VANILLA CHEMISTRY

by

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and approved by

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ABSTRACT OF THE DISSERTATION

Comparison and Integration of Analytical Methods for the Characterization of Vanilla Chemistry

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There is a need for an analytical method to establish a universal criterion for quality of cured vanilla bean. The chemistry of vanilla, one the world's most popular flavors is extremely complex. As such, no single analytical technique can fully characterize it. Commercially, vanilla is analyzed for many reasons including flavor/aroma quality, authenticity, geographic sourcing, concentration of vanillin and other major components, adulteration, contamination and quality defects. Furthermore, vanilla for analysis may be present in various forms such as alcoholic extracts or whole beans which present analytical challenges.

In this research an integrated analytical approach was used for the analysis of whole beans and extracts which spanned volatile and semi-volatile components. An improved high pressure liquid chromatography (HPLC) analytical method was developed

for routine analysis of vanillin and other phenolics in vanilla extracts resulting in lowered costs, greatly reduced analysis time and reduced solvent usage. A series of headspace concentration techniques including solid phase microextraction (SPME), headspace sorptive extraction (HSSE) and dynamic headspace were used to pinpoint a common sour, fermented off-odor quality defect in commercial Bourbon vanilla beans. Indicator compounds for microbial fermentation including fusel oil, microbial transformation products and depletion of flavor precursors were identified. Each headspace method has its own advantages and disadvantages. The sensitivity and selectivity of each method was manipulated to reveal individual clues of the off-odor. The combination of all three techniques gave detailed insight into the source of the defect. An improved direct thermal desorption gas chromatography mass spectrometry (DTD-GC-MS) method was developed to enhance resolution. Vanilla beans from Tanzania were analyzed for the first time using the improved DTD-GC-MS method and were found to contain very high concentrations of vanillin. Additionally, analysis of Bourbon, Indonesian, Ugandan, and Tahitian vanilla beans by the new DTD-GC-MS method revealed several new compounds in each that were not previously reported. Several novel compounds identified in a wild type vanilla bean previously by DTD-GC-MS including anisyl anistate, anisyl myristate and anisyl palmitate were synthesized to confirm the structure and GC-O was employed to evaluate their sensory properties.

Acknowledgements and Dedication

Research included in this dissertation on High Pressure Liquid Chromatography has been previously published by the Royal Society of Chemistry in Recent Advances in Food and Flavor Chemistry. **A rapid and efficient HPLC method for determination of vanillin and related phenolic components in vanilla extract using sub-2µm column technologies.** Stephen Toth, © The Royal Society of Chemistry, 2010.

Research included in this dissertation on the previously reported compounds in vanilla has been previously published by Wiley-Blackwell in Handbook of Vanilla Science and Technology. **Volatile Compounds in Vanilla.** Stephen Toth, Keun Joong Lee, Daphna Havkin-Frenkel, Faith C. Belanger and Thomas G. Hartman, © 2011 Blackwell Publishing Ltd.

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I hope this accomplishment in my life demonstrates to you what can be achieved with perseverance, dedication and hard work. You were my inspiration though this.

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1. Introduction

Vanilla is the most important and popular flavoring material in the world. It is also one of the most expensive flavoring materials. Recent data estimates the value at $80,000 USD per ton (Havkin-Frenkel and Belanger, 2008). Vanilla is the fully grown, edible fruit of tropical climbing orchids *Vanilla planifolia* Andrews (syn. *Vanilla fragrans* Ames.) and *Vanilla tahitensis* Moore. Harvested before fully ripe, the fruits are exposed to a multi-step curing process before bring extracted to produce the much desired flavoring and perfumery additive.

Vanilla is often described by the layperson as being "plain" or unremarkable due to the ubiquitous use of vanilla and vanilla flavored and fragranced products. However, specialists involved in the vanilla industry, flavorists and perfumers who can appreciate the complexity of vanilla know it is anything but "plain". Vanilla has a characteristic odor and taste as a result of a combination of hundreds of individual volatile and semi-volatile compounds working in harmony on the senses.

The use of vanilla is not limited to a simple flavor and fragrance additive. The main constituent of vanilla is vanillin. Vanillin has been shown to have medicinal, antimicrobial and anti-cancer properties. Current research in the medical field is investigating vanillin's properties as a cancer preventative agent (Ho, 2009), and it has been reported that vanillin is an effective inhibitor of red blood cell sickling in patients with sickle cell anemia (Bythrow, 2005). Vanillin has been shown to have antimicrobial activity both in the extract form as well as the plant and vine (Shanmugavalli, 2009).

The aroma of vanilla has even been shown to have a calming effect on newborns when familiarized with the odor (Rattaz, et. al, 2005).

This orchestra of compounds in vanilla has been the focus of research for years. As technology has evolved and produced more sensitive equipment and techniques, more has been learned about what makes vanilla, well, vanilla. From the original isolation of vanillin in 1858 by Gobley, to the Headspace Gas Chromatography research by Klimes and Lamparsky in 1976, to the Direct-Thermal-Desorption –Gas Chromatography-Mass Spectrometry by Hartman, et al. in 1992, as the instrumentation has progressed, so has our knowledge into the depths of vanilla.

1.1 History

Vanilla fragrans is native to the coast of eastern Mexico, and other parts of Central America. In the early 1500's, Hernando Cortez and his army of conquistadors arrived in Mexico to establish trade with the Aztecs. One of Spanish officers, Bernal Diaz, was the first to observe the Aztec emperor Montezuma drink "chocolatl" which was a beverage made from ground cacao seed and vanilla or "tlilxochitl"beans (Correll, 1953). Later, as legend would have it, Cortez himself would be served this very drink by Montezuma in a golden goblet. Upon returning to Spain with treasures of gold, silver and other valuable items, Cortez introduced vanilla to the rest of the world. The Spaniards began importing vanilla beans in the late 1500's and established factories for the processing of vanilla flavored chocolate.

Although not well documented, the vanilla plant was taken to Europe during the initial trading phase but did not flourish (Purseglove, 1981). In 1807, Mexican vanilla was reintroduced to the scientific community by the Marquis of Blandford and successfully cultivated in England by Charles Greville (Childers & Cibes, 1948). From the initial cultivation by Greville, cuttings were distributed to the botanical gardens in Paris and Antwerp. From the Antwerp collection, cuttings were further distributed to Indonesia, Reunion, and Mauritus (Purseglove, 1981). The plants grew well in their native tropical environment, but without the melipona bee, a natural pollinator for vanilla found in Mexico, the vines failed to produce fruit. It wasn't until 1836 when Charles Morren of Liege discovered an artificial means to pollinate the vanilla flower that vanilla beans were produced outside of its native Mexico. Later in 1841, a slave named Edmond Albius discovered method to hand-pollinate vanilla in Reunion which allowed the commercial production of vanilla to flourish. Immediately after vanilla was successfully pollinated and processed in Reunion, it was spread throughout the tropics and became an important fruit of commerce.

1.2 Botany

Vanilla is a climbing orchid native to tropical climates. There are 107 species of vanilla (Arditti, 2009), of which 54 are native to tropical America. Vanilla belongs to the orchid family, Orchidaceae, which is the largest family of flowering plants with over 700 genera and 20,000 species (Purseglove, 1981). Many orchid species are grown for their flowers, but vanilla is the only orchid that produces a commercial fruit. The most commonly grown vanilla of economic importance is *Vanilla planifolia* (Andrews)

Figure 1-1: Vanilla plant with pods

Source: http://giniann.files.wordpress.com/2006/03/vanilla

and *V. tahitensis* J.W. Moore.

V. planifolia is a fleshy, herbaceous perennial vine which can climb to height of 10-15m using its roots as an anchoring system. The roots are long, whitish and aerial with a diameter of approximately 2mm. The stem is long and cylindrical with a dark green color. The leaves are flat and fleshy and are typically 8-25cm long with a width of 2-8cm. The flowers of the vanilla vine are large (10cm in diameter) and pale green-yellow. There are three sepals which are 4-7cm long. The two upper petals are similar to the sepals, but are smaller in size. The lower petal, or labellum, acts as a lip. There is a concave sticky stigma which is separated from the stamen by the rostellum (Purseglove, 1981).

1.3 Climate & Soil Conditions

Vanilla thrives in warm, tropical climates from 25° north of the equator to 25° south. The preferred temperature range is 21-32°C with an average of 27°C. Annual rainfall requirements are 70-90 inches per year (Ranadive, 1994). The vanilla vines require a drier climate for 1-2 months in order to flower (Correll, 1953). The rainfall should be distributed evenly over the remainder of the year. The optimum location for vanilla vines is on gently sloping terrain. This allows adequate drainage of the soil which prevents fungal infection. The soil should be light and porous, neutral to slightly acidic, and be covered by a thick layer of mulch or humus (Guzman, 2004). Vanilla grows best at elevations up to 700m.

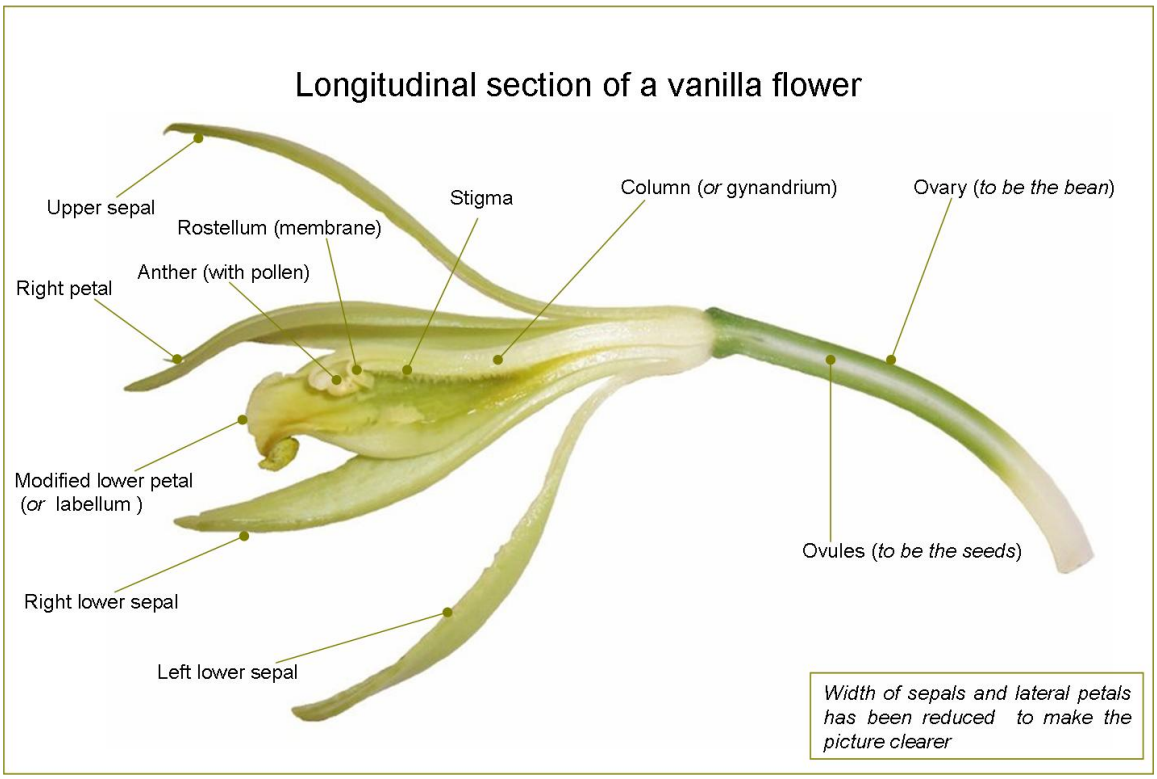

Figure 1-2: Longitudinal section of a vanilla flower

Source: www.vanillandflavors.com

1.4 Propagation, Pollination & Harvesting

Vanilla vines can be grown from seeds or propagated from cuttings of existing vines. Commercially, vanilla is propagated from stem cuttings around 30cm long as seeds can be difficult to germinate (Guzman, 2004). Cuttings of this length will produce flowers in 3-4 years if properly cared for. Cuttings of 8-10 nodes are preferred. After cuttings are planted, flowers begin to bloom in the third year. Once the vine reaches 7-8 year old, a maximum in flowering occurs. After 9-10 years of age, the vine does not flower and therefore, holds no commercial value (Correl, 1953; Purseglove, 1981). The life-cycle of the vines can but cut short by disease such as root rot, drought conditions, over pollination and improper soil conditions and vine supports (Childers, 1948).

Vanilla vines require support in order to thrive. Supports can be in the form of wooden or metal lattices, metal or porous cement poles, and more commonly, low branching trees. The trees are preferred in order to provide support as well as shade. In addition, certain fruiting trees can provide the vanillery with an additional source of income.

Healthy vanilla vines produce flowers once a year over a 1-2 month period. The flowering period is dependent on geographical location. Several conditions can affect whether or not the vanilla vine blossoms including drought, age, climate, training of the vines and pruning techniques. The flowers blossoms for only one day and pollination must take place during this time. The flower opens early in the morning and closes in early afternoon. The physical structure of the orchid flower prevents self-pollination and

therefore it must rely on natural pollinators or artificial pollination to produce fruit. In Mexico, the melipona bee has been identified as a natural pollinator along with hummingbirds. However, commercial vanilla vines are exclusively artificially pollinated by hand.

The hand pollination technique is performed by using a small sliver of wood. Holding the flower in one hand, the labellum in pushed down to expose the column. The stamen cap is set aside using the wooden stick which reveals the pollinia. Next the rostellum is pushed up under the stamen using the stick which allows the pollinia and stigma to touch each other using the thumb and forefinger. Once in contact, the pollen mass adheres to the stigma and the ovary is stimulated. If successful, the fertilized ovary becomes a small pod which grows to full size in 6-8 weeks. If unsuccessful, the flower will fall off the vine within a day (Purseglove, 1981).

Typically, only flowers on the lower side of the vine are pollinated. This allows the fruits to hang vertically during the growth period and produces straight beans which are considered to be of higher quality.

In 1836, flowers were successfully hand-pollinated in Belgium at the Liege Botanical Gardens by Charles Morren (Arditti, 2009). It was in 1841 when a slave named Edmund Albius is fabled to have developed the technique, independent of the scientific community and it publishing. There is a good possibility that several people

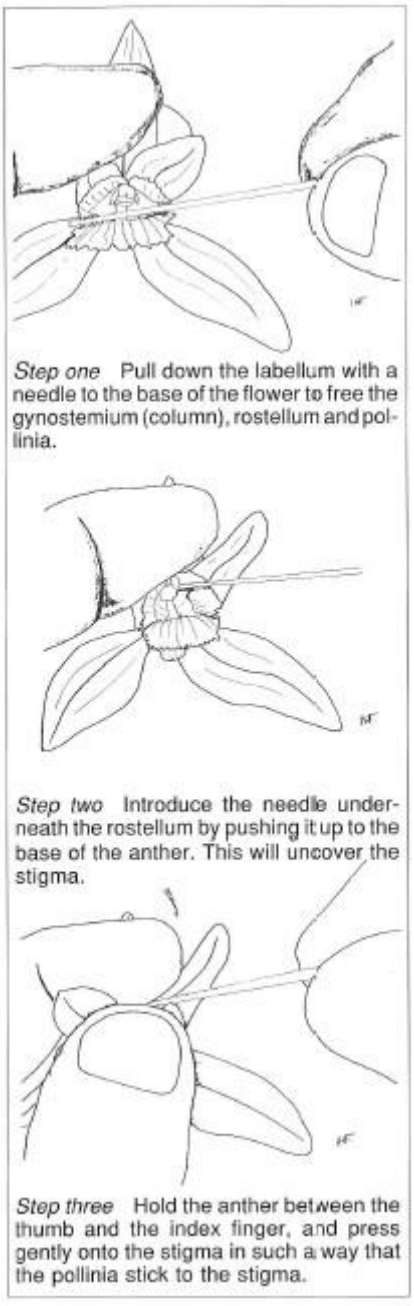

Figure 1-3: Illustration of hand-pollination technique

Source: (Fouche & Couman, 1992)

independently discovered the hand-pollination technique around the same time. As the world was trying to pollinate vanilla for commercial trade, many people were working on this at the same time. Simultaneous discoveries are very likely. However, whomever takes credit for the initial discovery, it cannot be overlooked that Edmund Albius's discovery on the island of Reunion enabled the vanilla industry to thrive on Reunion as well as other French establishments in the region such as Madagascar, Seychelles and Tahiti (Ecott, 2004).

The time between pollination and harvesting ranges from 6-9 months depending on geographic conditions. The beans are harvested by hand when they reach maturity. Some indications of bean maturity on the vine are a slight yellowing of the blossom end while the rest of the bean remains green, a color change of the bean from dark green to light green, a loss of shine or luster of the pod, and a visible and distinct line from one end of the fruit to the other (Purseglove, 1985; David, 1950). It is critical to harvest the fruits at the correct stage of maturity. If harvested too early, the beans will produce an inferior product. If harvested too late, the beans are likely to split during the curing. The curing process should commence within one week of harvesting to produce a high quality product.

1.5 Major Types of Vanilla

Vanilla beans differ by species, geographical region, curing processes as well as year-to-year variation of the same region and/or species. Given that certain regions

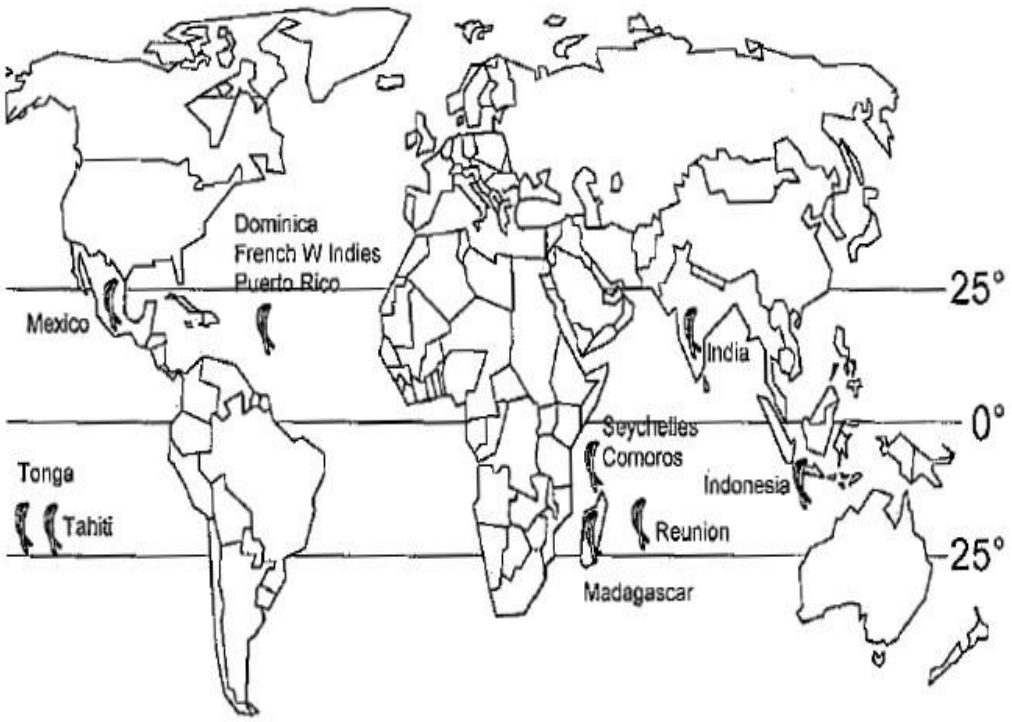

Figure 1-4: Geographical regions that commercially produce vanilla

Source: (Havkin-Frenkel, 1997)

produce beans of specific aromatic and flavoring character, each has its own application. There are two species of commercial interest: *Vanilla planifolia*, and *Vanilla tahitensis*

The majority of the vanilla bean trade worldwide can be linked back to either Indonesia or Madagascar. These two countries accounted for 81% of the 2009 vanilla crop as reported by the UN Food & Agriculture Organization (faostat.fao.org). Other minor producers of vanilla beans are India, China, Mexico, Tonga, Turkey, French Polynesia, Comoros and Uganda.

Mexican vanilla has a very rich flavor and aroma, but some consider it to lack depth. Bourbon vanilla on the other hand has depth to the flavor, but lacks in aroma. Both Mexico and the Madagascar areas cultivate *Vanilla planifolia*. The best quality vanilla beans come from Madagascar and are considered to be the best quality or "gold standard" (Krishnakumar, 2007)

Indonesian vanilla beans are also *Vanilla planifolia*, and contain a deep, rich flavor but are typically lower in vanillin content than Bourbon beans (Adedeji, 1993b).

Tahitian vanilla is obtained from *Vanilla tahitensis* and has a characteristic sweet, floral aroma, but lower vanillin content. This vanilla is typically used in flavorings and perfumery.

1.6 Quality

There are two major types of vanilla beans commercially available. One is called extraction grade, which the vanilla industry uses to make vanilla extract and the second one is called gourmet grade which is sold unprocessed. The extraction grade will have 18-25% moisture while the gourmet grade will have 20-30%. Vanilla tahitensis from Tahiti has an average moisture content of around 40%. The primary determinant of quality for vanilla beans is the aroma and flavor. Appearance is an important characteristic of quality. A high quality cured bean will have a pleasant aroma and flavor, proper moisture content (18-25%), dark chocolate coloring, acceptable length and girth, have an oily surface as well as being free of observable defects and mold (Adedeji, 1993b). Vanillin content, although extremely important in the commerce of vanilla beans, is not the sole deciding factor when it comes to considerations of quality. A high quality bean will have 2.0% or more vanillin content whereas a vanillin content lower than 0.2% is considered a low quality bean. Vanillin content in vanilla beans is only one indication of quality. There are many vanilla crops that have great overall flavors and low levels of vanillin.

1.7 Curing Techniques

The curing process is a succession of labor intensive events that convert green, ripe, odorless and bitter vanilla beans into dark-brown aromatic, tasteful beans over the course of approximately 5 months (Dunphy, 2009). Green, or immature, vanilla beans contain gluco-vanillin as well as other phenolic precursor compounds (Arana, 1945). Previous work has shown that prior to the curing process, the beans contain little, if any,

free vanillin (Arana, 1943). As the curing process progresses, vanillin is produced by the hydrolysis of gluco-vanillin via an enzymatic pathway involving beta-glucosidase (Balls and Arana, 1941).

The steps for curing vanilla beans vary with respect to which geographic region they are traditional to, the climate of the region and the available resources (Arana, 1945; Theodose, 1973). The variation of the process can have a significant impact on the aromatic profile, taste and overall quality of the beans. However, regardless of origin or heritage, all methods adhere to the same four traditional steps or stages: killing, sweating, drying and conditioning.

1.7.1 Killing, or wilting step

Once a ripe vanilla bean in harvested, it is necessary to stop the vegetative development and compromise the cell wall in order to release the various enzymes and begin the curing process (Ranadive, 1994). Upon disruption of the cell wall, the enzymes can begin reacting with their respective substrates which result in flavor and aroma development as well as dark brown coloring. Killing method include hot water scalding, sun exposure killing, oven killing, mechanical or scarring killing, freeze killing, and treatment with ethylene gas (Arana, 1944,1945; Jones and Vincente, 1948). Today, in practice, there are only three major killing procedures used: immersion in hot water (Madagascar, Indonesia, India, Uganda), placed in an oven (Mexico) and exposure to the sun (Papa New Guinea) (Dunphy and Bala, 2011).

1.7.2 Sweating step

Sweating of the killed vanilla beans involves the reduction of moisture through thermal drying techniques to reduce the onset of bacterial and fungal spoilage (Havkin-Frenkel, 1997), as well as trigger the enzymatic activity that produces the aroma, flavor and color associated with cured beans. The sweating process is carried out be either exposing the beans to sun for 6 hours per day, and then wrapping the beans in woolen blankets for the rest of the day, in sweat boxes, enclosed rooms or by using incubation ovens at 45°C (Arana, 1944, 1945). The process normally takes 7-10 days to complete. During the sweating procedure, enzymes that are contained within the vanilla bean are most active (Ranadive, 1994). This enzymatic activity is directly responsible for the release of vanillin from gluco-vanillin, and the color change of the beans from green to dark, chocolate brown (Balls and Arana, 1941). The sweating step is considered the most critical step of the curing process. Proper handling of the beans during this step will lead to a high quality cured bean, whereas improper handling including under/over-drying the beans, microbial contamination by dirty blankets or handlers, will result in a noticeable inferior product (Ranadive, 1994).

1.7.3 Drying step

At the end of the sweating process, the beans are rich in aroma, color and flavor but contain up to 70% moisture. This moisture must be reduced to prevent microbial spoilage and undesired enzymatic reactions. There are several methods used to dry the curing beans including oven drying, sun drying for 2-3 hours per day for 7-10 days and slow ambient temperature drying (Havkin-Frenkel, 1997). At the conclusion of the

Figure 1-5: Drying of vanilla beans in a Tahitian plantation

Source: www.vanillareview.com

drying process, the moisture content in the beans is lowered to 15-30% which is considered desirable (Jones and Vincente, 1948).

1.7.4 Conditioning step

The conditioning step is a process of aging the beans to further develop the aroma and flavoring. The beans are stored in closed boxes for a period of several months until the moisture and characteristic aroma are ideal. During this process, the vanilla bean develops additional "fullness", largely due to additional chemical reactions such as oxidation and hydrolysis (Ranadive, 1994). The duration of the conditioning process varies, but as a general rule it takes at least three months (Arana, 1944).

1.7.5 Mexican Curing Technique

The two most common method of wilting used in Mexico are sun killing and oven killing (Childers, 1953; Theodose, 1973). Sun killing is the oldest form of curing vanilla beans and begins by initially sorting the harvest beans by maturity and size. Next, the beans are spread out on woolen blankets in the sun for four to five hours. After the initial sunning, the beans are covered with the blankets but left out in the sun for an additional 3-4 hours. At night the beans are brought indoors and kept in wooden boxes. This process is repeated for six to eight days until all the beans achieve a dark brown color. The beans are then dried outdoors in wooden trays in the shade. The drying process can take as long as eight weeks (Ranadive, 1994). At this point, the beans are wrapped in waxed paper and stored in boxes for conditioning. This process lasts approximately three months.

Alternatively, the oven killing method in Mexico is more commercially used. This involves bundling the beans in large groups, dipping in water and then placing them in a large drying room called a ‘calorifico’. The temperature is raised in the room to 65-70°C by wood fired heaters and held for 36-48 hours. After this killing process, the beans undergo sweating, drying and conditioning steps as listed above.

1.7.6 Bourbon Curing Technique

This curing method was originally developed on the French island colony of Reunion, which was formally known as Bourbon. This method is carried out in Reunion, Madagascar and Comoros. The Bourbon method utilized a hot water scalding to kill the beans and fewer sweating steps are required versus the Mexican method.

The first step of the Bourbon curing method is to sort out the beans by size and maturity. Next, the beans are loaded into perforated baskets and immersed in hot water (65°C) for 2-3 minutes. Immediately after scalding, the beans are quickly wrapped in dark colored blankets and placed into sweating chests for 24 hours. The next day the beans are removed from the chests and sun-dried for 2-3 hours while still rolled up in the blankets to retain the moisture (Ranadive, 1994). This process of sweating and drying is repeated six to eight times, after which the beans are dried in large rooms. After the drying step, the beans are put into air-tight boxes for approximately three months.

1.7.7 Tahitian Curing Technique

In the Tahitian curing method, there is no artificial killing step as in the Mexican and Bourbon methods. The beans are allowed to reach maturity on the vine at which time they are harvested and placed into large piles. The piles are sweated overnight and this step is repeated for 2-3 weeks. The last step is drying which takes place outdoors in the open air (Ranadive, 1994).

1.7.8 Other Curing Techniques

There are several other documented curing methods. However none of them hold commercial significance as beans from these regions are not a main global source.

In the Guadeloupe method, the harvest beans are scarred by making cuts into the bean pod and then wrapping them blankets before sun exposure. These sweating and conditioning steps are similar to the Mexican curing method (Arana, 1945).

In the Guiana method, the beans are harvested and killed in the ashes of a fire. The heat shrivels the beans, at which time they are removed from heat, wiped with olive oil and then allowed to air dry (Ranadive, 1994).

In the Peruvian method, the beans are scalded similar to the Bourbon method, dried in the open air for approximately 20 days and then covered with castor oil (Adedeji, 1993b).

1.8 Improvements to Traditional Methods

Once Vanilla was established as a commercialized crop with world-wide demand, considerable scientific resources were put into optimizing the curing techniques to maximize the crops and increase the quality (Arana, 1944; Jones, 1948). Arana and his team at the Mayaguez USDA Federal Experimental Station in Puerto Rico produced several recommendations for vanilla cultivars. Additionally, other researchers appear to have been working on curing technique optimization and have also made contributions on the subject during the 1940's.

Arana (1944) and Jones and Vincente (1948) conducted research on the curing various curing methods. The conclusions were that the Bourbon scalding method was the most preferred method. The Guadeloupe method of scarification did produce a better quality product in a shorter timeframe, but were susceptible to mold and had decreased flexibility. Mold and other diseases within the beans were a large priority to the researchers as this could destroy a very labor intensive agricultural product. Arana (1945) states that non-uniformity in the drying and sweating steps, sun drying, the use of dirty blankets for sweating, and improper ventilation in the curing rooms all lead to the susceptibility of the crop to mold and disease. Control of the moisture during these steps is paramount. Arana (1944) found that using electric ovens at 45°C to sweat and dry the vanilla beans was more uniform, produced a superior product and had less occurrences of mold. Later, Rivera and Hageman (1950) concluded that 38°C produced a better product.

More recently, researchers have attempted to reduce the time of curing, or increase the quality of the product using specialized laboratory techniques Sreedhar and his team have shown that pretreating the vanilla beans during accelerated curing conditions with naphthalene acetic acid or ethrel can increase the vanillin production three fold by the tenth day of curing (Sreedhar et al, 2007). Marquez et al. investigated the different killing techniques by monitoring β-glucosidase enzyme inactivity. Their research concluded 38°C and pH 6.5 to be the optimum. (Marquez, 2008). Pardio et al performed enzyme assisted ethanolic extracts of vanilla beans. Their work has shown an increase in the major flavor compounds in an aged extract versus a non-enzymatic ethanolic extract control. (Pardio, 2009).

1.9 Types & Grades

After the conditioning process, cured beans are sorted into grades according to their length. The length of the bean is the primary determinant of the value of the beans. Secondary determinants are shape, color, moisture content and aroma. The grading system of cured beans varies according to geographical region. The beans are typically identified as belonging to one of three categories: unsplit beans, split beans and cuts. Cuts are beans that had some sort of defect removed from them by cutting it out. Usually this defect is mold or disease. Small beans, broken beans and mis-shapened beans are typically combined with the cuts (Purseglove, 1981). The nomenclature for Mexican beans is (from highest quality to lowest): prime, good to prime, fair and ordinary. Bourbon beans are classified as: prime, firsts, seconds, thirds, fourths, and foxy splits (Guzman, 2004).

1.10 Disease & Spoilage of Beans

Vanilla vines and beans and subject to many diseases, most of which are caused by poor drainage, above average rainfall and excessive shade. The most serious disease is Anthracnose, *Calospora vanillae Massee*, which causes the vine to lose its fruits. Another problematic disease caused by the fungus *Fusarium oxysporum* f. sp. *vanillae*, also known as *Fusarium batatis* var. *vanilla Tucker*, which causes the rotting of the stem and roots and subsequently kills the plants (Ranadive, 1994; Hernandez-Hernandez, 2011). Correll (1953) lists in detail the following diseases that attack vanilla: *Phytophthora parasitica* Dast., causing bean rot; *Glomerella vanillae* (Zimm.) Petch, attacking roots; *Vermicularia vanillae* Delacr. in Mauritus; *Gleospriu, vanillae* Cooke in Columbia; *Macrophoma vanillae* Averna in Brazil; and *Pestalozzia vanillae* Averna, also in Brazil. (Purseglove, 1981) More recently, several chapters have been written about the current viral and fungal diseases which attack vanilla (Hernandez-Hernandez, 2011; Grisoni, 2010; Tombe, 2010)

1.11 Manufactured products

In the United States, a large majority of the vanilla is processed as an alcoholic extract for use in flavoring, both commercially and domestically. In Europe, the majority is processed as whole or powdered vanilla (Ranadive, 1994). Vanilla is used in a wide variety of food including: ice cream, confectionary products, chocolate, beverages, and desserts just to name a few.

1.11.1 Vanilla Extracts

The vanilla extract is an aqueous alcoholic extract containing soluble organics from the vanilla beans. This extract is highly regulated by the Food and Drug Administration. Vanilla extract is the only flavoring material with a U.S. FDA standard of identity. The Code of Federal Regulations, Title 21, section 169.175 defines a vanilla extract as: "the solution in aqueous ethyl alcohol of the sapid and odorous principles extractable from vanilla beans." The finished vanilla extract contains 35% ethanol and must include the soluble compounds from 1 part by weight in 10 parts by volume of the solvent. The extracts can be prepared by direct solvent extraction, dilution of oleoresins or dilution of concentrated vanilla extracts.

1.11.2 Vanilla Flavoring

Vanilla flavoring or flavor is exactly like vanilla extract, but the amount of ethanol is less than 35% by volume.

1.11.3 Concentrated Extracts and Flavorings

Vanilla extract of more than 4 fold strength are not practical from a processing standpoint using only solvent extraction. To achieve higher concentrations, part of the solvent is removed under high vacuum. This does create a higher strength extract, but the product lacks some of the fullness of a lower strength extract as some of the volatile organics are lost in the solvent stripping procedure.

1.11.4 Tinctures

There are two types of vanilla tinctures commercially available. One is for perfumery use and is prepared by the extraction of vanilla beans in denatured alcohol. The finished product contains 90% alcohol. The second type of tincture is for pharmaceutical use and is made by extracting vanilla beans in aqueous alcohol in a ratio of 1 part vanilla bean by weight to 10 parts solvent by volume and contains added sugar. The final concentration is at least 38% (Purseglove et al, 1981).

1.11.5 Vanilla Powder/Sugar

Vanilla powder is made by mixing ground vanilla beans, concentrated vanilla extract or vanilla oleoresin with sugar or maltodextrin.

1.11.6 Absolutes

Vanilla absolute is a very highly concentrated solvent extract used for perfumery. The extraction solvent can be hydrocarbons or chlorinated hydrocarbon, or a combination of the two. Further, beans from different regions and species can be extraction together to give a desired character to the absolute. Some absolutes are prepared by supercritical carbon dioxide extraction (Ranadive, 1994).

1.11.7 Oleoresins

This is the dark brown, semi-solid, viscous end product of an aqueous ethanolic extraction followed by solvent removal under vacuum. Finely cut vanilla beans are extracted using 50% (v/v) aqueous ethanol at around 50°C. Previous to 1986, the US

Bureau of Alcohol Tobacco and Firearms allowed the use of either ethanol or isopropanol (Ranadive, 1994). After 1986, only aqueous ethanol was allowed. Some research on using higher solvent concentrations was down by Cowly who concluded that increased alcoholic content reduced the oleoresin yield (Cowly, 1973).

For industrial use, the oleoresins are diluted to the desired strength and added as-is. The oleoresins do not contain the full aroma or flavor of the original vanilla bean as some of the lighter organic molecules are lost to the solvent stripping process.

1.12 Biochemistry of Vanilla During Curing

Many chemical and biochemical reaction take place during the curing process which yield a variety of flavor-active and aroma-active compounds that contribute to the overall flavor and fragrance of the cured bean (Perez-Silva, 2006). It has been well documented that the main biochemical reaction that occurs during the curing process is the hydrolysis of glucovanillin (Balls, 1941). Research by Arana (Arana, 1943) and Balls and Arana (Balls and Arana, 1941) have shown the significance of beta-glucosidase on vanillin formation during the curing process. Their research proved that vanillin is produced by the hydrolytic enzymatic transformation of glucovanillin and that additional hydrolysis of other glucosides present in the bean produce other distinct aroma and flavor compounds.

Arana has shown that the glucovanillin content of the vanilla beans gradually increase over time as the fruits mature on the vine. Additionally, the glucovanillin is not

uniform throughout the bean. The majority of the concentration is focused at the blossom end, with the least amount being present nearest the stem (Arana, 1943, 1944).

In depth studies of vanilla beans has shown that the glycosylated flavor precursors are found within the bean interior whereas the enzymes that catalyze the reactions for flavor and aroma production are located mostly in the outer wall of the fruit (Havkin-Frenkel, 2005). The curing process serves as a facilitator to bring the precursors and enzymes in contact. There are many aroma compound precursors found in vanilla beans as glycosides including p-hydroxybenzaldehyde, p-cresol, p-hydroxybenzoic acid, vanillic acid, vanillyl alcohol and p-hydroxybenzyl alcohol (Dignum, 2002).

During the curing process, only about half of the potential vanillin, both free vanillin and glucovanillin, present in the beans actually survive the multi-step procedure and is found in the cured bean (Gatfield, 2007). The initial steps of the curing process, which include killing and sweating, produce a rapid decrease in the concentration of glucovanillin as it is hydrolyzed to vanillin. This inversely produces a rapid increase in the concentration of vanillin. The fate of approximately half of potential vanillin can be accounted for by losses due to sublimation (Frenkel, 2006), or possible participation in the Maillard reaction.

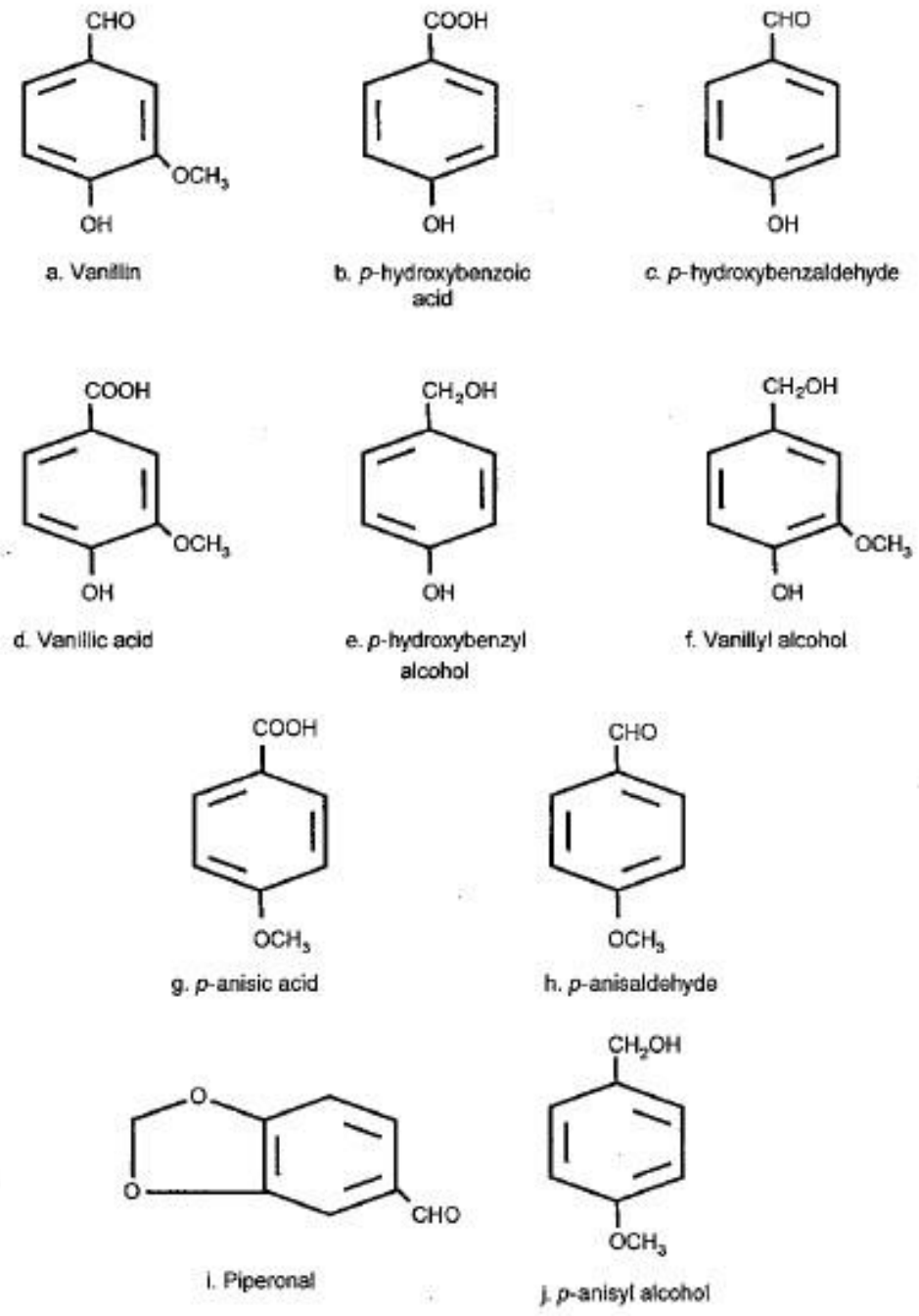

Figure 1-6: Chemical structures of major compounds for the flavoring and aroma of vanilla

Source: (Guzman, 2004)

1.13 Aroma & Flavor Characteristics

The characteristic aroma and flavor of each vanilla species can be easily tasted and smelled. These differentiating attributes can be exploited to obtain desired flavors and scents.

1.13.1 Bourbon

Bourbon vanilla (*V. planifolia*) which is grown in Madagascar, Reunion, Comoros Islands, and the Seychelles has been described as: sweet, creamy, rich, tobacco-like, woody, animal, balsamic and spicy (Ranadive, 1994).

1.13.2 Mexican

Mexican vanilla (*V. planifolia*) which is native to Mexico has been described as: sharp, pungent, sweet, spicy, and incomplete with reference to Bourbon vanilla (Ranadive, 1994).

1.13.3 Java

Java vanilla or Indonesian vanilla (*V. fragrans*) which is native to the Indonesian islands has been described as: less sweet and creamy than Bourbon vanilla, strong woody, and smoky (Ranadive, 1994).

1.13.4 Tahitian

Tahitian vanilla (*V. tahitensis*) which is grown in Tahiti has been described as: perfumy, flowery, fragrant, and heliotropin-like (Ranadive, 1994).

1.13.5 Guadeloupe

Guadeloupe vanilla (*V. pompona*) which is native to Central America has been described as: perfumy, floral, sweet, and lacks body (Ranadive, 1994).

1.13.6 Indian

Indian vanilla (*V. planifolia*) which is grown in India has been described as: less sweet and creamy than Bourbon vanilla, lack balsamic note, pungent, sour (Ranadive, 2011)

1.13.7 Papau New Guinea (PNG)

PNG vanilla (*V. tahitensis*) which is grown in Papua New Guinea has been described as: weak flowery, perfumed, anisic, overall weak (Ranadive, 2011).

2. Literature Review

2.1 Volatile Constituents of Vanilla

Identification of volatile and semi-volatile compounds in vanilla beans and vanilla extracts has evolved over time as the complexity and sensitivity of the instrumentation has improved. Initially isolated and identified by Gobley in 1858, vanillin was synthesized and confirmed by Tiermann and Haarmann in 1874 (Tiermann and Haarmann, 1874). In 1909, anisyl alcohol, anisaldehyde and anisic acid were discovered in Tahitian vanilla (Walbaum, 1909). Later work in Tahitian vanilla by Gnadinger (1925) revealed aromatic cinnamic esters. Several other minor discoveries of compound occurred during the early twentieth century. However, it wasn't until the chromatographic work of Klimes and Lamparksy in that an additional 170 components were identified using gas chromatography (Klimes and Lamparsky, 1976). The advent of direct thermal desorption headspace gas chromatography – mass spectrometry analysis, pioneered by Hartman in 1992 and Adedeji in 1993 allowed detailed identification of volatile and semi-volatile components in raw vanilla beans that had not been previously been possible (Hartman, 1992; Adedeji, 1993a,b). Further, an in-depth study of vanilla beans using direct thermal desorption gas chromatography mass spectrometry by Lee in 2006 gave compelling insight into vanilla beans from various geographical locations as well as different species (Lee, 2006).

2.2 Previously published compounds

The following table is an exhaustive list of all known volatile and semi-volatile compounds that have been identified in vanilla beans and extract in the publically accessible literature. It should be noted that omission of a compound from a particular species or geographical region does not imply that it does not occur naturally, but it was not reported in the specific cited analysis. Also, certain species have not been studied as deeply as others. The number of compounds identified per species or sample is most likely a consequence of analytical methodology used rather than complexity of the vanilla being studied. The contents of this list have been published in the 'Handbook of Vanilla Science and Technology' (Toth,*et al*., 2011) and is reproduced here with permission from Wiley-Blackwell publishing.

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Balli	Java	Mexican	Tonga	Costa Rican	Jamaican
ACIDS										
(9Z)-Octadec-9-enoic acid	oleic acid	112-80-1	13 (6), 110-356 (8)	280 (8)			16 (2), 203 (8)	239 (8)		
2,2-dimethyl-3-butenoic acid		10276-09-2				48 (1)				
2,3-dihydroxypropyl ester 9,12-octadecadienoic acid	glyceryl monolinoleate									
2-heptenoic acid	hept-2-enoic acid	18999-28-5					2 (2)			
2-hydroxy-2-(3-hydroxy-4-methoxyphenyl)acetic acid	**isovanillylmandelic acid**	3695-24-7	3 (8)							
2-hydroxybenzoic acid	salicylic acid	69-72-7								
2-hydroxypropanoic acid	lactic acid	50-21-5								
2-methyl-1-(1,1-dimethyl)-2-methylpropanoic acid			32 (1)							
2-methylpropanoic acid	isobutyric acid	79-31-2					2 (2)			
2-propenoic acid	**acrylic acid**	79-10-7	22 (8)							
3,4-dimethoxybenzoic acid	**veratric acid**	93-07-2								
3-methylbutanoic acid	isovaleric acid	503-74-2				62 (1)	4 (2)	34 (8)		35 (1)
4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	110-586 (1), 440 (6), 246-825 (8)	112 (1), 186 (8), (20)	520-869 (1)	270 (1)	994 (1), 1315 (2), 887 (8)	439 (1), 472 (8)	787 (1)	830 (1)
4-hydroxybenzoic acid	p-hydroxybenzoic acid	99-96-7	6 (1), 28 (6)	136 (1), 2478 (8), (20), (28)	194 (1)		218 (1), 255 (2)	49 (1)	310 (1)	47 (1)
4-methoxybenzoic acid	p-anisic acid	100-09-4	69-267 (1), 49 (6)	6471 (1), 87370 (3), 4129 (8), (19), (24)			10 (2), 22 (8)			
4-methyl-2-oxovaleric acid	**isopropyl pyruvic acid**	816-66-0		97 (8)			3 (8)			
4-methyl-3-cyclohexene-1-carboxylic acid, methyl ester						36 (1)				
4-oxopentanoic acid	levulinic acid	123-76-2	14 (1), 120-126 (8)	460 (8)	52 (1)			2 (1), 209 (8)		
4-phenoxybenzoic acid		2215-77-2		25 (1)						
9,12-octadecadienoic acid	linoleic acid	60-33-3	59-509 (1), 135 (6), 117-411 (8)	190 (1), 509 (8)	367-561 (1)	66 (1)	70 (1), 225 (2), 408 (8)	54 (1), 451 (8)	142 (1)	1421 (1)
9-hexadecanoic acid	palmitoleic acid	373-49-9	38 (8)	26 (8)			6 (2), 8 (8)	24 (8)		
a,d-dimethyltetronic acid										
acetic acid	ethanoic acid	64-19-7	1130-2348 (1), 151 (6), 1078-1347 (8)	1515 (1), 2844 (8)	937-1153 (1)	955 (1)	361 (1), 124 (2), 683 (8)	135 (1), 1820 (8)	1587 (1)	1613 (1)
benzene propanoic acid	hydrocinammic acid	501-52-0					4 (2)			
benzoic acid	dracylic acid	65-85-0	38 (6)				3 (2)			
butanoic acid	butyric acid	107-92-6					<1 (2)			
3-phenyl-2-propenoic acid	cinnamic acid	140-10-3	114 (1)		52-342 (1)		3 (2)			
cyclohexaneacetic acid	**cyclohexylacetic acid**	5292-21-7	13 (8)							
decanoic acid	capric acid	334-48-5								
dodecanoic acid	lauric acid	143-07-7	6-20 (8)		583 (1)	74 (1)	2 (2)	8 (8)		
formic acid	methanoic acid	64-18-6	130 (1), 62 (6), 314-342 (8)	471 (8)			1904 (1), 98 (8)	72 (1), 319 (8)	168 (1)	185 (1)
heptadecanoic acid	margaric acid	506-12-7	8-27 (1), 30 (6), 27 (8)	20 (8)	4 (1)	35 (1)	110 (1), 6 (2), 7 (8)	18 (1), 10 (8)		35 (1)
heptanoic acid	enanthic acid	111-14-8					2 (2)			
hexadecanoic acid	palmitic acid	57-10-3	23-54 (1), 258 (6), 279-754 (8)	383 (1)	60-516 (1)	28 (1)	127 (1), 127 (2), 475 (8)	18 (1), 568 (8)		30 (1)
hexanoic acid	caproic acid	142-62-1	23 (8)				102 (1), <1 (2), 39 (8)			
hydroxy acetic acid	glycolic acid	79-14-1								
m-anisic acid, methyl ester				76 (8)						
methoxyacetic acid	methoxyethanoic acid	625-45-6								
nonanoic acid	pelargonic acid	112-05-0	95 (1), 31 (6)				130 (1), 16 (2)			
octadecanoic acid	stearic acid	57-11-4	42-587 (1), 39 (6), 41-99 (8)	15 (1), 108 (8)	11-80 (1)	95 (1)	67 (1), 14 (2), 47 (8)	60 (1), 81 (8)	17 (1)	97 (1)
octanoic acid	caprylic acid	124-07-2				157 (1)	6 (2)			
pentadecanoic acid	pentadecylic acid	1002-84-2	38-254 (1), 24 (6), 12-29 (8)	34 (1), 33 (8)	69-89 (1)	192 (1)	43 (1), 13 (2), 11 (8)	37 (1), 17 (8)	8 (1)	22 (1)
pentanoic acid	valeric acid	109-52-4					2 (2)			
propanoic acid	ethanecarboxylic acid	79-09-4	18-199 (1)	67 (1)			2 (2)	6 (8)		
spirohexane-1-carboxylic acid, ethyl ester				26 (1)				34 (1)		
tetradecanoic acid	myristic acid	544-63-8	22-343 (1), 12 (6), 45-51 (8)	29 (1), 80 (8)	102-336 (1)	66 (1)	42 (1), 12 (2), 16 (8)	15 (1), 20 (8)	20 (1)	78 (1)
ALCOHOLS										
(2E)-3,7-dimethylocta-2,6-dien-1-ol	nerol	106-25-2								
(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	24 (1), 9 (6), 25-54 (8)	65 (1), 225830 (3),9515 (8), (9) (19) (22) (24) (28)			71 (1), 2 (2), 7 (8)	17 (1), 49 (8)		
(7,7-dimethyl-4-bicyclohept-3-enyl)methanol	myrtenol	6712-78-3								
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	phytol	150-86-7	6 (8)				7 (8)			
1-(4-methoxyphenyl)-2-methyl-3-buten-1-ol				15 (8)						
1,2,3,4-butanetetraol	erythritol	149-32-6				43 (1)				
1,2,3-propanetriol	glycerol, glycerine	56-81-5	978 (1)							
1,2-cyclohexanediol		931-17-9							40 (1)	
1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	29 (6)							
1,2-dimethoxybenzene	**veratrol**	91-16-7					18 (8)			

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
1,2-propanediol	propylene glycol	57-55-6					<1 (2)			
1,3-butanediol	b-butylene glycol	107-88-0	219-326 (1), 47 (6), 55-65 (8)		238-334 (1)	44 (1)	301 (1), 95 (8)	570 (8)		227 (1)
1,3-cyclohexanediol, trans-	**hexahydroresorcinol**	504-01-8	84 (8)	110 (8)						
1,4-butanediol	tetramethylene glycol	110-63-4	89-259 (1)				226 (1)			
1-methoxy-2-(4-methoxyphenyl)methylbenzene				245 (1)						
1-octen-3-ol	octenol, vinyl hexanol	3391-86-4					<1 (2)			
1-phenyl-1,2-butanediol			130 (1)		63 (1)					
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	alpha-terpineol	98-55-5								
2,2,4-trimethyl-3-penten-1-ol								64 (1)		
2,2-dimethylpentan-1-ol	**neoheptanol**	2370-12-9	103 (8)							
2,3-butanediol	dimethylene glycol, 2,3-butyleneglyco	24347-58-8			951 (1)		17 (2), 174 (8)			
2,4-dimethyl-1-heptanol							20 (1)			
2,6-dimethoxy-4-methylphenol	**4-methyl syringol**	6638-05-7								
2,6-dimethyl-4-ethyl-4-heptanol								43 (1)		
2-acetoxy-1-propanol				8 (8)						
2-butyne-1,4-diol						17 (1)				
2-cis-9-octadecenyloxyethanol			184 (1)	28 (1)						
2-ethylcyclobutanol		35301-43-0	14 (8)							
2-methoxy-3-methylbutane					1091 (1)					
2-methoxy-4-(2-propenyl)-phenol	eugenol	97-53-0							15 (1)	
2-methoxy-4-methylphenol	creosol	93-51-6					4 (2)			
2-methoxy-4-prop-2-enylphenol	**engenol, caryophyllic acid**	97-53-0								
2-methoxy-p-cresol	**2-Hydroxy-5-methylanisole**	93-51-6	3 (8)				12 (8)	10 (8)		
2-methoxyphenol	guaiacol, methylcatechol	9009-62-5	19 (6), 220-322 (8)				9 (2), 3 (8)	77 (8)		
2-methyl-butan-1-ol		137-32-6								
2-octen-4-ol	butyl propenyl carbinol	20125-81-9	25 (6)							
2-phenylethanol	phenylethyl alcohol	60-12-8					1 (2)			
3-(hydroxymethyl) phenol		620-24-6	20 (1)		30 (1)	202 (1)				
3,4-Dimethoxybenzyl alcohol	**veratryl alcohol**	93-03-8								
3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	nerolidol	40716-66-3			110 (1)					
3,7-Dimethyl-2,6-octadien-1-ol	geraniol	106-24-1								
3,7-dimethyl-6-octen-1-ol	citronellol, dihydrogeraniol	106-22-9								
3-7-dimethylocta-1,6-dien-3-ol	linalyl alcohol, linalool	78-70-6								
3-methyl-2-buten-1-ol	prenol	556-82-1								
3-methyl-2-propylpentan-1-ol			47 (1)	102 (1)				7 (1)		243 (1)
3-methyl-butan-1-ol	isopentanol	6423-06-9								
3-methylhexan-2-ol		2313-65-7	178 (8)		14 (1)			171 (1)		
3-methylpentan-1-ol		589-35-5								68 (1)
3-methylphenol	**cresol**	108-39-4								
3-phenyl-2-propen-1-ol	cinnamyl alcohol	104-54-1					<1 (2)			
3-phenylpropan-1-ol	benzene propanol	122-97-4					<1 (2)			
4-(3-hydroxy-1-propenyl)-2-methoxyphenol	coniferyl alcohol	458-35-5								
4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol	498-00-0		(10)			84 (2)	12 (8)		
4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	29 (6), 15 (8)	(10)			65 (2)	4 (8)		
4,5-octanediol		22607-10-9								
4-butoxy-1-butene										138 (1)
4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	318 (8)				1 (2), 33 (8)			
4-ethenylphenol	4-vinyl phenol	2628-17-3					2 (2)			
4-ethenylphenol	p-vinyl phenol	2628-17-3								
4-Ethyl-1,3-benzenediol	4-ethylresorcinol	2896-60-8	48 (1)		156 (1)				173 (1)	
4-ethyl-2-methoxyphenol	p-ethyl guaiacol	2785-89-9	33 (6)							
4-Methoxybenzenepropanol		5406-18-8								
4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	terpinen-4-ol	562-74-3								
4-methylphenol	p-cresol	106-44-5				27 (1)	3 (2)			
9,12-octadecadien-1-ol	linoleyl alcohol	506-43-4		64 (1)						
9-octadecen-1-ol	oleyl alcohol	143-28-2		28 (1)						
benzene-1,4-diol	hydroquinone, quinol	123-31-9			143 (1)					
but-2-yne-1,4-diol		110-65-6	10 (8)							
docosan-1-ol	behenyl alcohol	661-19-8	44 (1)		201 (1)					
dodecan-1-ol	lauryl alcohol	112-53-8								
dodecylcyclohexanol					33 (1)					
heptacosan-1-ol		2004-39-9	140-1353 (8)	216 (8)			223 (8)	641 (8)		
heptadecan-1-ol	heptyl alcohol	111-70-6								
heptan-2-ol	amyl methyl carbinol	543-49-7		612 (8)	40 (1)					
hexacosan-1ol		506-52-5	222 (8)	50 (8)			186 (8)			

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
hexadecan-1-ol	cetyl alcohol, palmityl alcohol	36653-82-4	38 (6)							
hexan-1-ol	hexyl alcohol	111-27-3								
hexan-2-ol		626-93-7	175-629 (8)					867 (8)		
hydroxytricosane			14-31 (1)							
methyl-2-vinylethyl carbinol							6 (8)			
nonan-2-ol		628-99-9								
nonan-3-o1		624-51-1	277 (1)					107 (8)	109 (1)	
o-catechol			85-2224 (1)	27 (1)	1966 (1)	60 (1)				133 (1)
octacosan-1-ol	montanyl alcohol	557-61-9	1289 (8)	427 (8)			2224 (8)	556 (8)		
octan-1-ol	octyl alcohol, capryl alcohol	111-87-5					1 (2)			
pentan-1-ol		71-41-0								
pentan-2-ol	amyl alcohol	6032-29-7	95 (6)							
phenol	phenyl alcohol, benzenol	108-95-2	362-553 (1), 28 (6), 26-161 (8)	143 (1), 23 (8)	263 (1)	201 (1)	190 (1), 2 (2)	14 (8)	95 (1)	210 (1)
phenylmethanol	benzyl alcohol	100-51-6					3 (2)			
tetradecan-1-ol	myristyl alcohol	112-72-1	13 (6)							

ALDEHYDES

(2E,4E)-deca-2,4-dienal		80-56-8	209 (1), 25 (6)	34 (1)		83 (1)	1 (2), 168 (8)			112 (1)
2,2-dimethylpent-4-enal		5497-67-8				97 (1)				
2,6,6-trimethylcyclohexene-1-carbaldehyde	beta-cyclocitral	52844-21-0								
2-hydroxy-2-methylpropanal		20818-81-9	13 (1)							
2-hydroxybenzaldehyde	salicylic aldehyde	90-02-8								
2-methylbut-2-enal	2-methyl-2-butenal	6038-09-1					140 (1)			
2-oxopropanal	pyruvaldehyde	78-98-8	44 (1)							
3,3-dimethylhexanal									227 (1)	
3,4-dihydroxybenzaldehyde	**protocatechualdehyde**	139-85-5		23 (8)						
3,5-Dimethyoxy-4-hydroxybenzaldehyde										
3-methoxybenzaldehyde	m-anisaldehyde	591-31-1		954 (1)						
3-methylbutanal	Isovaleraldehyde	590-86-3	37-48 (1), 27 (6)	32 (1), 13 (8)	18-370 (1)	459 (1)	150 (1)	36 (1), 37 (8)	45 (1)	6 (1)
3-methylpentanal	3-methyl valeraldehyde	15877-57-3	24-378 (1), 15-242 (8)	368 (1), 6 (8)	27-222 (1)	566 (1)	104 (1), 2 (8)	44 (1), 13 (8)	81 (1)	36 (1)
4-hydroxy-3,5-dimethoxybenzaldehyde	syringic aldehyde	134-96-3	26-457 (1), 10 (6), 25 (8)		113 (1)			12 (8)		
4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	15896-17465 (1), 19400 (6), 16674-22435 (8)	6797 (1), (7), 5798 (8)	15227-20054 (1)	3413 (1)	9296 (1), 19118 (2), 22757 (8)	12193 (1), 10429 (8)	17613 (1)	12271 (1)
4-hydroxybenzaldehyde		123-08-0	790-950 (1), 1040 (6), 1396-1414 (8)	1387 (1), 812 (8), (22) (27)	772-1114 (1)	55 (1)	635 (1), 873 (2), 1549 (8)	288 (1), 467 (8)	765 (1)	604 (1)
4-methoxybenzaldehyde	anisaldehyde	123-11-5	12 (8)	25030 (3), 329 (8) (9) (24) (29)						
acetaldehyde	ethanal	75-07-0								
benzaldehyde		100-52-7								
dec-2-enal	2-decenal	3913-81-3					2 (2)			
decanal		112-31-2	4-5 (8)							
hept-2-enal	2-heptenal	29381-66-6					2 (2)			
hexacosanal		26627-85-0	417 (8)	32 (8)			677 (8)	166 (8)		
hexanal	hexanaldehdye	66-25-1	277 (1)		389 (1)		206 (1)	724 (1)		356 (1)
nonanal	nonanaldehyde	124-19-6	1901 (1), 53 (6), 26-52 (8)		110 (1)					
octacosanal		22725-64-0	61 (8)							
octadec-9-enal	octadecenyl aldehyde	5090-41-5			54 (1)					
pentanal	valeraldehyde	110-62-3								

ALKANES

1,3,5-trimethylcyclohexane		1839-63-0					11 (8)			
10-methyleicosane		54833-23-7	25 (6)							
11-decylheneicosane		55320-06-4	23 (1)	60 (1)	14-44 (1)	33 (1)	38 (1)			
2,2,4,6,6-pentamethylheptane	isododecane	31807-55-3	16 (1)	23 (1)						
2,2,4-trimethylpentane	**isooctane**	540-84-1		136 (8)						
2,3,3,4-tetramethylpentane		16747-38-9				62 (1)				
2,6,10,15-tetramethyl heptadecane				12 (1)		1587 (1)				
2,7,10-triethyldodecane					21 (1)					
2-cyclohexyleicosane		4443-56-5	10 (1)							

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Baili	Java	Mexican	Tonga	Costa Rican	Jamaican
3-ethyl-2-methylpentane		68333-81-3					56 (1)			
3-ethyl-3-methylpentane		1067-08-9					32 (1)			
4-ethylheptane		2216-32-2						22 (1)		
4-ethyltetradecane		55045-14-2	33 (1)							
6-ethyl-2-methyldecane		62108-21-8				25 (1)				
decane		73138-29-1								
docosane		629-97-0	7-11 (1), 22 (6), 31 (8)	36 (1), 111(8)	14 (1)	687 (1)	67 (1), 29 (8)	34 (1), 7 (8)	64 (1)	25 (1)
dodecane		94094-93-6								
heneicosane		629-94-7	23 (6)							
hentriacontane		630-04-6		359 (8)						
heptacosane		593-49-7	38-204 (8)	26 (8)			162 (8)	79 (8)		
heptadecane		629-78-7								
hexacosane		630-01-3	24 (6), 16-90 (8)	18 (8)			49 (8)	30 (8)		
hexadecane		544-76-3								
hexane		92112-69-1				263 (1)			432 (1)	
hexatricontane		630-06-8	78-114 (1)	126 (1)	32 (1)	17 (1)	15 (1)		26 (1)	43 (1)
eicosane	eicosane	112-95-8	39 (6)							
nonacosane		630-03-5	25-203 (8)	79 (8)			188 (8)	197 (8)		
nonane		111-84-2								
octacosane		630-02-4				37 (1)				
pentacosane		629-99-2	98-404 (8)	91 (8)			20 (2), 235 (8)	158 (8)		
pentadecane		629-62-9								
pentatriacontane		630-07-9	47-108 (1)	34 (1)	13-170 (1)	242 (1)		43 (1)	23 (1)	
tetracosane		646-31-1	13 (6), 42-198 (8)	30 (8)			91 (8)	61 (8)		
tetradecane		629-59-4								
tricosane		638-67-5	89 (8)							
tricosane		638-67-5	101-585 (1), 26 (6), 314 (8)	325 (1), 57 (8)	38-191 (1)	249 (1)	54 (1),16 (2), 172 (8)	29 (1), 119 (8)	36 (1)	224 (1)
undecane		1120-21-4								
x-decane (branched)										
ALKENES										
(1R,4aS,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	gamma-cadinene	39029-41-9								
(1S,4aS,8aR)-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	alpha-muurolene	10208-80-7								
1,2,3-trimethoxy-5-methylbenzene	**3,4,5-trimethoxytoluene**	6443-69-2								
1,2,3-trimethylbenzene		526-73-8								
1,2-dimethoxybenzene	veratrole	91-16-7								
1,3-dimethylbenzene	xylene	1330-20-7								
1-cyclohexen-1-ylethanone	**1-acetylcyclohexene**	932-66-1	9 (8)							
1-ethyl-4-methylbenzene	p-ethyl toluene	622-96-8								
1-methyl-4-(6-methylhept-5-en-2-yl)benzene	alpha-curcumene	644-30-4								
1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohexene	beta-bisabolene	495-61-4								
1-methyl-4-prop-1-en-2-ylcyclohexene	limonene	5989-27-5								
1-methyl-4-propan-2-ylbenzene	cymene	99-87-6								
1-methyl-4-propan-2-ylcyclohexa-1,3-diene	alpha-terpinene	99-86-5								
1-Tricosene		18835-32-0	73 (8)					25 (8)		
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	217 (1), 514-592 (8)	22 (1), 363 (8)	24-83 (1)	487 (1)	392 (8)	148 (8)		
3-eicosene		74685-33-9			195 (1)	453 (1)				
3-methylidene-6-propan-2-ylcyclohexene	beta-phellandrene	555-10-2								
4,7,7-trimethylbicyclo[3.1.1]hept-3-ene	alpha-pinene	80-56-8								
5-eicosene		74685-30-6	16-42 (1)			16 (1)		49 (1)	30 (1)	16 (1)
7,7-dimethyl-4-methylidenebicyclo[3.1.1]heptane	beta-pinene	127-91-3								
7-methyl-3-methylideneocta-1,6-diene	myrcene	123-35-3								
9-tricosene		27519-02-4	17-29 (1)		23-83 (1)	2345 (1)	18 (1)	38 (1)	25 (1)	86 (1)
benzene	benzol	71-43-2								
docos-1-ene	**docosene**	1599-67-3	14-44 (8)	23 (8)			14 (8)	16 (8)		
ethenylbenzene	styrene	100-42-5								
ethyl benzene	ethylbenzol, phenylethane	100-41-4								
heptacos-1-ene	**heptacosene**		12-42 (8)	35 (8)			36 (8)	52 (8)		
hexacos-1-ene	**hexacosene**	18835-33-1	32 (8)				21 (8)	3 (8)		

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Baili	Java	Mexican	Tonga	Costa Rican	Jamaican
hexadec-1-ene	**hexadecene**	629-73-2		92 (1)						
methylbenzene	toluene	108-88-3								
napthalene	naphthalin, antimite	91-20-3								
non-4-ene		68526-55-6						11 (1)		
nonacos-1-ene			79-423 (8)	211 (8)			621 (8)	194 (8)		
pentacos-1-ene			39 (6), 24-180 (8)				142 (8)	69 (8)		
propylbenzene	1-phenylpropane, isocumene	103-65-1								
tricosene			38 (6)							
x-dodecene										
x-eicosene										
x-tetradecene										
ESTERS										
(1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl) acetate	bornyl acetate	76-49-3	(4)							
(4-formyl-2-methoxyphenyl) acetate	acetovanillin	881-68-5	27 (6)							
(4-methoxyphenyl)methyl 4-hydroxy-3-methoxybenzoate	**anisyl vanillate**									
(4-methoxyphenyl)methyl (E)-3-phenylprop-2-enoate	anisyl trans-cinnamate			530 (3)						
(4-methoxyphenyl)methyl (Z)-3-phenylprop-2-enoate	anisyl cis-cinnamate			150 (3)						
(4-methoxyphenyl)methyl 2-hydroxybenzoate	**anisyl salicylate**									
(4-methoxyphenyl)methyl (3,4-dohydroxybenzoate)	**anisyl protocatechuate**									
(4-methoxyphenyl)methyl acetate	anisyl acetate	104-21-2		233 (1), (9)						
(4-methoxyphenyl)methyl anisate	anisyl anisate			6640 (3)						
(4-methoxyphenyl)methyl formate	anisyl formate	122-91-8	(4)	25 (1)			2 (2)			
(4-methoxyphenyl)methyl hexadecanoate	**anisyl stearate**			472 (8)						
(4-methoxyphenyl)methyl hexanoate	**p-anisyl hexanoate**	6624-60-8								
(4-methoxyphenyl)methyl hydroxybenzoate	anisyl 4-hydroxybenzoate			7400 (3)						
(4-methoxyphenyl)methyl octadec-9-enoate	**anisyl oleate**			2720 (8)						
(4-methoxyphenyl)methyl octadec-9,12-dienoate	**anisyl linoleate**									
(4-methoxyphenyl)methyl octadec-9,12,15-trienoate	**anisyl linolenate**									
(4-methoxyphenyl)methyl octadecanoate	**anisyl petadecanoate**									
(4-methoxyphenyl)methyl petadecanoate	**anisyl palmitate**			160 (8)						
(4-methoxyphenyl)methyl propenoate	**anisyl acrylate**									
(4-methoxyphenyl)methyl tetradecanoate	**anisyl myristate**									
(4-propan-2-ylphenyl) acetate	**4-isopropylphenyl acetate**									
[(1R,4S,6R)-1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl] acetate	isobornyl acetate	125-12-2	(4)							
[(2S)-2,3-dihydroxypropyl] (9Z,12Z)-octadeca-9,12-dienoate	2,3-dihydroxypropyl linoleate	2277-28-3				62 (1)				
[(E)-3-phenylprop-2-enyl] (E)-3-phenylprop-2-enoate	cinnamyl cinnamate	122-69-0								
[(E)-3-phenylprop-2-enyl] benzoate	cinnamyl benzoate	5320-75-2								
2-(4-methylcyclohex-3-en-1-yl)propan-2-yl acetate	alpha-terpinyl acetate	80-26-2	(4)							
2,3-dihydroxypropyl acetate	**glycerolmonoacetate**	93713-40-7	75-2204 (1), 287-444 (8)	27 (1)			100 (1), 251 (8)	266 (8)		251 (1)
2-acetyloxyethyl acetate	**ethylene diacetate**	111-55-7								
2-ethylhexyl (E)-3-(4-methoxyphenyl)prop-2-enoate	**2-Ethylhexyl-4-methoxycinnamate**	5466-77-3								
2-ethyl-trans-bicyclohexyl methyl 14-methylpentadecanoate			118 (1)		7 (1)	34 (1)				
2-hydroxyethyl acetate	ethylene glycol acetate	65071-98-9						29 (1)	44 (1)	
2-methylpropyl pentanoate	isobutyl valerate	10588-10-0								
2-pentanal propanoate								7 (1)		
2-phenethyl formate		104-62-1	(4)							
2-phenylethylacetate		103-45-7								
3,7-dimethyloct-6-enyl 2-methylpropanoate	citronellyl isobutyrate	97-89-2	(4)							
3,7-dimethylocta-1,6-dien-3-yl acetate	linalyl acetate	115-95-7	(4)							
3-hydroxypropyl (Z)-octadec-9-enoate	3-hydroxypropyl oleate			54 (1)						
3-hydroxypropyl prop-2-enoate	**2-hydroxypropyl acrylate**	25584-83-2								
3-methylbutyl 2-hydroxybenzoate	isoamyl salicylate	87-20-7	(4)							
4-hexen-1-ol acetate							33 (1)			
7-methyl-4-octanol acetate			7 (1)		28 (1)					52 (1)
butyl hexanoate	butyl caproate	626-82-4	(4)							
butyl pentanoate	butyl valerate	591-68-4								
diethyl benzoate										
ethenyl formate	vinyl formate	692-45-5			3095 (1)			17 (1)		
ethyl 2-(4-hydroxy-3-methoxyphenyl)acetate	**ethyl homovanillate**	60563-13-5	4 (8)							

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
ethyl 2-hydroxy-2-methylbutanoate	ethyl-2-methyl butyrate	77-70-3								
ethyl 2-hydroxybenzoate	ethyl salicylate	118-61-6	(4)							
ethyl 2-hydroxypropanoate	ethyl lactate	97-64-3								
ethyl 2-methoxyacetate	ethyl methoxyacetate	3938-96-3								
ethyl 4-hydroxy-3-methoxybenzoate	**ethyl vanillate**	617-05-0						15 (8)		
ethyl 4-oxopentanoate	ethyl levulinate	539-88-8								
ethyl acetate		141-78-6								
ethyl hexadecanoate	ethyl palmitate	628-97-7								
ethyl hexanoate	ethyl caproate	123-66-0								
ethyloctadeca-9,12,15-trienoate	ethyl linolenate	1191-41-9					14 (2)			
hexyl 2-hydroxybenzoate	hexyl salicylate	6259-76-3	(4)							
hexyl acetate	methamyl acetate	142-92-7	178 (8)	12 (8)			21 (8)			
hexyl acetate		142-92-7								
hexyl butanoate		2639-63-6	(4)							
methoxymethyl acetate		4382-76-7					218 (1)			
methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0	21 (6)							
methyl 10,13-octadecadienoate			16-70 (1)	27 (1)	39 (1)	179 (1)	70 (1)		10 (1)	39 (1)
methyl 11-octadecenoate						136 (1)				
methyl 2-hydroxyacetate	methyl glycolate	96-35-5								
methyl 2-hydroxybenzoate	methyl salicylate	119-36-8					<1 (2)			
methyl 2-hydroxypropanoate	methyl lactate	547-64-8								
methyl 2-oxopropanoate	methyl pyruvate	600-22-6	11 (1)	66 (1)	134-369 (1)		57 (1)		120 (1)	252 (1)
methyl 2-phenylacetate		101-41-7								
methyl 3-methoxybenzoate	methyl m-anisate	5368-81-0		218 (1)						
methyl 4-(2-hydroxyethoxy)benzoate										
methyl 4-(hydroxymethyl)benzoate		6908-41-4	36 (6)							
methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	15 (8)							
methyl 4-hydroxybenzoate		99-76-3	13-77 (1)		275 (1)				10 (1)	65 (1)
methyl 4-methoxybenzoate	methyl anisate	121-98-2		(9)						
methyl acetate	Tereton	79-20-9	24 (1), 84 (6),15 (8)							
methyl acrylate	**methyl prop-2-enoate**	96-33-3	99-103 (8)	110 (8)			69 (8)	13 (8)		
methyl benzoate		93-58-3								
methyl dodecanoate	methyl laurate	111-82-0								
methyl heptadecanoate	methyl margarate	1731-92-6								
methyl heptanoate		106-73-0								
methyl hexadecanoate	methyl palmitate	112-39-0	13 (6), 4 (8)					23 (8)		
methyl hexanoate	methyl caproate	106-70-7								
methyl icosanoate	methyl arachidate	1120-28-1					6 (1)			
methyl nonanoate		1731-84-6								
methyl pentadecanoate		7132-64-1								
methyl pentanoate	methyl valerate	624-24-8								
methyl tetradecanoate	methyl myristate	124-10-7								
methyl-2-(4-hydroyphenoxy) benzoate										
methyl-3-phenylprop-2-enoate	methyl-trans-cinnamate	1754-62-7	27-33 (8)	32 (1), 24 (8), (9)			1 (2)	4 (8)		
methyl-4-hydroxybenzoate			19 (8)							
methyl-8-methyldecanoate			21 (1)				21 (1)	6 (1)		
pentyl 2-hydroxybenzoate	pentyl salicylate	2050-08-0	(4)							
pentyl acetate	n-amyl acetate	628-63-7								
phenylmethyl (E)-3-phenylprop-2-enoate	benzyl cinnamate	103-41-3								
phenylmethyl acetate	benzyl acetate	140-11-4								
phenylmethyl benzoate	benzyl benzoate	120-51-4								
phenylmethyl butanoate	benzyl butyrate	103-37-7								
phenylmethyl formate	benzyl formate	104-57-4								
prop-2-enyl octadecanoate	allyl stearate	6289-31-2			77 (1)					
propan-2-yl acetate	isopropyl acetate	108-21-4			53 (1)					
propan-2-yl pentanoate	isopropyl valerate	18362-97-5								
propyl 4-hydroxybenzoate	**propyl paraben**	94-13-3	83 (1), 24 (8)		134 (1)					
propyl pentanoate	propyl valerate	141-06-0								

ETHERS

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
1,2-dimethoxyethane	ethylene glycol dimethyl ether	110-71-4	264 (1)							
1-methoxyhexane	**methyl hexyl ether**	4747-07-3	7 (8)							
1-methoxypropane	**methyl propyl ether**	557-17-5	15 (8)							
1-propoxypropane	propyl ether	111-43-3		74 (8)					321 (1)	
2-ethoxypropane	isopropyl ethyl ether	625-54-7	40 (1)		115 (1)		30 (8)		219 (1)	
2-methoxypropane	methyl isopropyl ether	598-53-8	275-388 (1)							
3-ethenoxyprop-1-ene	vinyl allyl ether	3917-15-5	3 (8)					10 (1)		
3-methoxypentane	1-Ethylpropyl methyl ether	36839-67-5			4 (1)					
4-(ethoxymethyl)-2-methoxyphenol	vanillyl ethyl ether	13184-86-6								
4-(ethoxymethyl)phenol	p-hydroxybenzyl ethyl ether	57726-26-8								
4-(methoxy)-2-methoxyphenol	vanillyl methyl ether						<1 (2)			
anisole	methoxybenzene	100-66-3								
phenoxybenzene	diphenyl ether	101-84-8								
	anisyl ethyl ether			15040 (3)						
	anisyl methyl ether			800 (3)						
	dianisyl ether			3130 (3)						
	isopentyl methyl ether			76 (8)			4 (8)			
	methyl cyclobutyl ether									
	p-cresyl isopropyl ether									
	p-hydroxybenzylmethyl ether		17 (6)							

KETONES

(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	alpha-ionone	9066-80-2		410 (3)						
(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	beta-ionone	79-77-6		1000 (3)						
(E)-oct-3-en-2-one		1669-44-9								
1-(2,4,6-trimethylphenyl)ethanone	2,4,6-trimethylacetophenone		42 (1), 69 (6)			47 (1)		52 (1)		
1-(2,4-dihydroxyphenyl)ethanone	2,4-dihydroxyacetophenone	89-84-9	4-22 (8)		80-233 (1)	21 (1)				
1-(2-hydroxy-5-methylphenyl)ethanone	o-acetyl-p-cresol	1450-72-2	24 (6), 221 (8)	87 (8)				26 (8)		
1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	35-82 (1), 37 (6), 12-23 (8)			70 (1)	309 (1), 14 (2), 6 (8)	8 (1), 9 (8)	95 (1)	59 (1)
1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2503-46-0	2 (8)				167 (1)	32 (8)		
13-methyl-oxacyclotetradecane-2-11-dione			27 (1)							
1-hydroxyheptan-2-one										
1-hydroxypentan-2-one										
1-hydroxypropan-2-one	hydroxy acetone, pyruvic alcohol	116-09-6	63-216 (8)		587 (1)	546 (1)	53 (8)	8 (8)		
1-methoxypropan-2-one	methoxyacetone	5878-19-3	1391 (1), 5 (8)	23 (1), 13 (8)	44-177 (1)	6 (1)		15 (1)	1 (1)	146 (1)
1-phenylethanone	acetophenone	98-86-2								
2,3,3,4-tetramethylcyclobutan-1-one										
2,4-dimethylpentan-3-one	**diisopropyl ketone**	565-80-0								
2-butan-2-ylcyclopentan-1-one			34 (8)				12 (8)			
2-hydroxy-3-methylcyclopent-2-en-1-one		80-71-7						3 (1)		
2-methylpentan-2-one			117-130 (1)	21 (1)	8-332 (1)	5 (1)	361 (1)		45 (1)	
3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one	**b-hydroxypropiovanillone**									
3-hydroxybutan-2-one	**2-acetoin**	513-86-0					15 (2)	10 (8)		
3-methyl-3-decen-5-one								49 (1)		
3-methylbut-3-en-2-one		814-78-8							252 (1)	
3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	21 (1)			67 (1)				
3-methylcyclohex-2-en-1-one		1193-18-6	34-154 (1)	77 (1)	102 (1)	40 (1)		49 (1)	90 (1)	139 (1)
3-methylcyclohexan-1-one		625-96-7	68 (1)							
3-methylcyclopentan-1-one		6672-30-6	159-500 (1), 207 (8), 25 (6)	10 (1)	106-132 (1)	117 (1)	181 (1)	225 (1)	211 (1)	34 (1)
3-methylpentan-2-one	**sec-butyl-methyl ketone**	565-61-7								
3-Oxabicyclo[3.2.0]heptane-2,4-dione		7687-27-6					62 (8)			
3-penten-2-one	methyl propenyl ketone	625-33-2								
4-(4-hydroxy-3-methoxyphenyl)butan-2-one	zingerone,vanillyl acetone	122-48-5	46 (6)					9 (8)		
4-(4-hydroxyphenyl)-2-butanone	raspberry ketone	5471-51-2	31 (6)							
4,4-dimethyl-2-oxethanone					79 (1)					
4-acetyl-2-hydroxy-5-methylbenzene									246 (1)	
4-butoxy-3-methylbutan-2-one							149 (1)			
4-methylene-2-oxethanone							95 (1)			
5-hydroxy-2,3-dimethyl-2-cyclopentene-1-one										

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
5-hydroxyheptan-2-one										
6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	502-69-2	23 (6)			40 (1)		6 (8)		
butan-2-one	methyl ethyl ketone	78-93-3			35 (1)					
butane-2,3-dione	diacetyl	431-03-8	6-22 (8)	25 (8)				19 (1), 2 (8)		
cycloheptanone	suberone	502-42-1	35 (1)				6 (8)			
cyclohexane-1,3-dione	dihydroresorcinol	504-02-9	69 (1)							
cyclohexane-1,4-dione	tetrahydroquinone	637-88-7					10 (1)			
cyclohexanone		108-94-1	24 (8)	413 (8)			194 (8)			
cyclopent-4-ene-1,3-dione		930-60-9	16-49 (8)	10 (8)						
decan-2-one	octyl methyl ketone	693-54-9								
di(phenyl)methanone	**diphenyl ketone**	119-61-9								
hentriacontene-2,4-dione			966 (8)	481 (8)			621 (8)			
heptacosane-2,4-dione			3696-5110 (8)							
heptacosene-2,4-dione			6272 (8)	15897 (8)			55475 (8)	11073 (8)		
heptan-2-one	amyl methyl ketone	29308-56-3								
heptan-4-one	dipropyl ketone	123-19-3							110 (1)	
heptane-2,4,6-trione	diacetyl acetone	626-53-9	57 (6)							
hex-5-en-2-ol		626-94-8	58 (1)							
hexan-2-one	propyl acetone	591-78-6								
hexane-2,3-dione		3848-24-6								
hexane-2,4-dione		3002-24-2	162-236 (1)		9-137 (1)	56 (1)				149 (1)
nonacosene-2,4-dione			145 (8)	6154 (8)			15434 (8)	2572 (8)		
nonan-2-one	heptyl methyl ketone	821-55-6								
octa-4,6-dien-3-one										
octan-2-one	hexyl methyl ketone	27457-18-7								
pentacosane-2,4-dione			107 (8)				197 (8)	36 (8)		
pentacosene-2,4-dione										
pentan-2-one	**ethyl acetone**	27157-48-8	26 (8)							
pentane-2,3-dione	**acetyl propionyl**	600-14-6								

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1-(1H-pyrrol-2-yl)ethanone	2-acetyl pyrrole	1072-83-9								
1,3,7-trimethylpurine-2,6-dione	caffeine	95789-13-2		12 (3)						
1,3-benzodioxole-5-carbaldehyde	heliotropine, piperonal	120-57-0		(11) (23) (26) (27) (29)						
1,4-dimethylpiperazine	**Lupetazine**	106-58-1								
1-furan-2-ylethanone	acetyl furan, 2-furl methyl ketone	80145-44-4	42-152 (1), 4-45 (8)	138 (1), 22 (8)	75 (1)			33 (1)		
1-furan-2-ylpropan-1-one	2-propionylfuran	3194-15-8						20 (1)	202 (1)	
1H-pyrrole-2,5-dione, ethyl-4-methyl							2 (2)			
2-(hydroxymethyl)-5-hydroxy-4H-pyran-4-one					80-156 (1)		169 (1)	8 (1)	159 (1)	
2,2,4,5-tetramethyl-1,3-dioxolane										
2,2'-bi-1,3-dioxolane					263 (1)					
2,3-dihydro-1-benzofuran	coumaran	496-16-2	69-125 (1), 114 (6), 21-128 (8)	95 (1), 21 (8)	63-286 (1)		366 (1)	113 (1), 7 (8)	195 (1)	52 (1)
2,3-dihydro-2,5-dimethylfuran			57 (1)	110 (1)	21 (1)		256 (1)		195 (1)	
2,5-dimethyl furfural										
2,6,6-trimethyl-10-methylidene-1-oxaspiro[4.5]dec-8-ene	vitispirane	65416-59-3		280 (3)						
2,6-dimethyl-3(2H)-benzo-furanone				12 (1)			17 (1)		18 (1)	
2,6-dimethyl-4-pyranone			10-72 (1)	216 (1)						
2-butyltetrahydrofuran			40 (1)		40-78 (1)	254 (1)				
2-ethyl-1,3-dioxolane							3 (1)			
2-furancarboxylic acid methyl ester			14 (6)							
2-hydroxy-5-methyl furan										
2-pentylfuran	2-amyl furan	64079-01-2					39 (1)			50 (1)
2-propylfuran		4229-91-8	216-263 (1)						520 (1)	368 (1)
3,4-dimethylfuran-2,5-dione	**dimethylmaleic anhydride**	766-39-2	150-737 (8)	1604 (8)			493 (8)	75 (8)		
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one			14-3268 (1)	3601 (1)	84-4076 (1)	1780 (1)	3886 (1)	1416 (1)	2350 (1)	2476 (1)
3,5-dimethyl-2,4-(3H,5H)-furandione			202-419 (1)	14 (1)	42-375 (1)	200 (1)				171 (1)
3,7-dimethylpurine-2,6-dione	theobromine	83-67-0		40 (3)						
3H-pyran-2,6-dione	**glutaconic anhydride**		18 (8)	10 (8)				3 (8)		
3-hydroxy-2-methylpyran-4-one	maltol	118-71-8	84-176 (1), 42-66 (8)	54 (8)	23 (1)	415 (1)	10 (8)	12 (1), 200 (8)	78 (1)	119 (1)

IUPAC Name	Synonyms	CAS #	Bourbon	Tahitian	Bali	Java	Mexican	Tonga	Costa Rican	Jamaican
3-hydroxy-4,4-dimethyl-dihydro-2(3H)-furanone						66 (1)				
3-hydroxy-4,4-dimethyloxolan-2-one	pantolactone	79-50-5	51 (6)				1 (2)			
4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	10 (6)							
4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one	dihydroactinidiolide	19432-05-4		230 (3)						
4-hydroxy-2,5-dimethylfuran-3-one	**furaneol, strawberry furanone**	3658-77-3	36-99 (8)	86 (8)			11 (8)	10 (8)		
4-methyl-2H-furan-5-one		22122-36-7	20 (8)	15 (8)						
5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	76330-16-0	460 (6), 1511-6707 (8)	6878 (8)			596 (8)	3287 (8)		
5-(hydroxymethyl)-tetrahydro-2-furanol										
5,6-dihydro-2H-pyran-2-carboxaldehyde					102 (1)					
5-ethylfuran-2-carbaldehyde	**5-ethylfurfural**	23074-10-4								
5-hydroxy-2-(hydroxymethyl)pyran-4-one	**kojic acid**	67-99-2	5-18 (8)	12 (8)			6 (8)	63 (8)		
5-isopropyldihydro-3(2H)furandione						20 (1)				
5-methyl-3H-furan-2-one		591-12-8	22-272 (1)	143 (1)	100-360 (1)	66 (1)	179 (1)	114 (1)	230 (1)	80 (1)
5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	534-646 (1), 24 (6), 21-105 (8)	217 (1), 26 (8)	430-606 (1)	421 (1)	36 (1), 3 (8)	19 (1), 93 (8)	406 (1)	134 (1)
5-methyl-tetrahydro-2-furan-methanol			340 (1)		79-748 (1)					
5-pentyloxolan-2-one	coconut aldehyde	104-61-0								
6-undecyloxan-2-one	hexadecalactone	7370-44-7				45 (1)				
dihydro-5-isopropyl-3(2H)-furanone			23 (8)							
dihydroxydihydromaltol			335 (6)							
furan-2,5-dione	maleic anhydride	24937-72-2	4 (1)							
furan-2-carbaldehyde	furfural	98-01-1	560-672 (1), 54 (6), 131-645 (8)	838 (1), 598 (8)	449-1176 (1)	560 (1)	1039 (1), <1 (2), 63 (8)	74 (1), 147 (8)	882 (1)	542 (1)
furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	214-625 (1), 15 (6), 225-354 (8)	362 (1), 41 (8)	230-347 (1)	268 (1)	468 (1), 45 (8)	73 (1), 174 (8)	664 (1)	406 (1)
furan-2-ylmethyl acetate	furfuryl acetate	623-17-6								
furan-2-ylmethyl benzoate	furfuryl benzoate	34171-46-5								
furan-3-ylmethanol	**3-furanmethanol**	4412-91-3								
furfuryl hydroxy methyl ketone										
hydroxydihydromaltol			2912-4805 (8)	4556 (8)			2131 (8)	3836 (8)		
hydroxymaltol			12 (6)							
methyl 5-oxotetrahydro-2-furancarboxylate			31 (8)				25 (8)			
methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	611-13-2	12-69 (1)	19 (1), 104 (8)			369 (1), 9 (8)			
methyl pyridine-3-carboxylate	methyl nicotinate	93-60-7								
oxolan-2-one	gamma-butyrolactone	96-48-0	3-20 (8)				<1 (2), 6 (8)			
oxolan-2-ylmethyl acetate	tetrahydro-2-furfuryl acetate	637-64-9			49 (1)					
pyran-4-one		29943-42-8	130 (1)	219 (1), 8 (8)						
tetrahydro-4,4,6,6,-tetramethyl-2H-pyran-2-one						60 (1)	52 (8)			
tetrahydroxymethylfurfuryl alcohol			17 (6)							
thiophene	thiofuran	110-02-1								
trans-2,3-dimethyl-tetrahydro-2-furanol			4 (1)		628 (1)					

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pomponia* origin unknown	*V. pomponia* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
ACIDS													
(9Z)-Octadec-9-enoic acid	oleic acid	112-80-1			852 (8)	63 (8)	156-834 (8)	529 (8)	253 (8)	3639 (8)	291 (8)	71-2288 (8)	1033 (8)
2,2-dimethyl-3-butenoic acid		10276-09-2											
2,3-dihydroxypropyl ester 9,12-octadecadienoic acid	glyceryl monolinoleate						47 (8)						
2-heptenoic acid	hept-2-enoic acid	18999-28-5											
2-hydroxy-2-(3-hydroxy-4-methoxyphenyl)acetic acid	**isovanillylmandelic acid**	3695-24-7					189 (8)						
2-hydroxybenzoic acid	salicylic acid	69-72-7	(7) (12)				108 (8)					30-2971 (8)	
2-hydroxypropanoic acid	lactic acid	50-21-5	(7)										
2-methyl-1-(1,1-dimethyl)-2-methylpropanoic acid													
2-methylpropanoic acid	isobutyric acid	79-31-2	(15)										
2-propenoic acid	**acrylic acid**	79-10-7					6-30 (8)						11 (8)
3,4-dimethoxybenzoic acid	**veratric acid**	93-07-2								52 (8)			
3-methylbutanoic acid	isovaleric acid	503-74-2	(7) (15)			40 (8)	47-138 (8)	86 (8)		9 (8)	9 (8)	30-441 (8)	
4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	(12) (15) (20) (25) (31)	(20)	1011 (8)	915 (8)	440-1963 (8)	1077 (8)	1158 (8)	390 (8)	577 (8)	244-726 (8)	1360 (8)
4-hydroxybenzoic acid	p-hydroxybenzoic acid	99-96-7	(12) (20) (28)	(20)								30 (8)	121 (8)
4-methoxybenzoic acid	p-anisic acid	100-09-4	(19) (24)		39 (8)		79 (8)				33 (8)	23-7945 (8)	1488 (8)
4-methyl-2-oxovaleric acid	**isopropyl pyruvic acid**	816-66-0								112 (8)		130 (8)	174 (8)
4-methyl-3-cyclohexene-1-carboxylic acid, methyl ester													
4-oxopentanoic acid	levulinic acid	123-76-2	(15)		282 (8)	148 (8)	54-213 (8)	184 (8)	128 (8)	173 (8)	131 (8)	85-239 (8)	
4-phenoxybenzoic acid		2215-77-2										7 (8)	
9,12-octadecadienoic acid	linoleic acid	60-33-3			3805 (8)	7072 (8)	211-942 (8)	857 (8)	586 (8)	7600 (8)	252 (8)	89-14484 (8)	2609 (8)
9-hexadecanoic acid	palmitoleic acid	373-49-9				300 (8)	47-269 (8)			65 (8)		95 (8)	61 (8)
a,d-dimethyltetronic acid					177 (8)						17 (8)		
acetic acid	ethanoic acid	64-19-7	(7) (15)		1647 (8)	1135 (8)	849-1855 (8)	1343 (8)	1713 (8)	2178 (8)	365 (8)	1488-2417 (8)	2667 (8)
benzene propanoic acid	hydrocinammic acid	501-52-0											
benzoic acid	dracylic acid	65-85-0	(15) (31)										
butanoic acid	butyric acid	107-92-6	(7)										22 (8)
3-phenyl-2-propenoic acid	cinnamic acid	140-10-3	(19)								79 (8)		
cyclohexaneacetic acid	**cyclohexylacetic acid**	5292-21-7											
decanoic acid	capric acid	334-48-5	(7) (16)										
dodecanoic acid	lauric acid	143-07-7	(7)			69 (8)	21-42 (8)	73 (8)	3 (8)	80 (8)	36 (8)	11-77 (8)	
formic acid	methanoic acid	64-18-6	(7)		507 (8)	293 (8)	168-550 (8)	730 (8)	599 (8)	289 (8)	198 (8)	340-1280 (8)	635 (8)
heptadecanoic acid	margaric acid	506-12-7			39 (8)	51 (8)	47-224 (8)	37 (8)	62 (8)	124 (8)	11 (8)	63-300 (8)	54 (8)
heptanoic acid	enanthic acid	111-14-8	(9)									60 (8)	
hexadecanoic acid	palmitic acid	57-10-3			859 (8)	2054 (8)	658-2020 (8)	1314 (8)	790 (8)	4240 (8)	323 (8)	350-5795 (8)	1659 (8)
hexanoic acid	caproic acid	142-62-1	(7) (9)										
hydroxy acetic acid	glycolic acid	79-14-1	(7)										
m-anisic acid, methyl ester							34 (8)	6 (8)				11-56 (8)	
methoxyacetic acid	methoxyethanoic acid	625-45-6	(7)							11 (8)			
nonanoic acid	pelargonic acid	112-05-0	(9)										
octadecanoic acid	stearic acid	57-11-4			421 (8)	544 (8)	108-474 (8)	191 (8)	98 (8)	885 (8)	58 (8)	34-1094 (8)	1758 (8)
octanoic acid	caprylic acid	124-07-2	(7) (9) (16)										
pentadecanoic acid	pentadecylic acid	1002-84-2			59 (8)	143 (8)	51-178 (8)	101 (8)	45 (8)	243 (8)	10 (8)	12-385 (8)	92 (8)
pentanoic acid	valeric acid	109-52-4											
propanoic acid	ethanecarboxylic acid	79-09-4					6-68 (8)	30 (8)	8 (8)			3-11 (8)	
spirohexane-1-carboxylic acid, ethyl ester													
tetradecanoic acid	myristic acid	544-63-8	(7)		59 (8)	269 (8)	58-240 (8)	467 (8)	44 (8)	191 (8)	19 (8)	25-293 (8)	193 (8)
ALCOHOLS													
(2E)-3,7-dimethylocta-2,6-dien-1-ol	nerol	106-25-2	(7)										
(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	(7) (13)	(19) (21)	410 (8)	70 (8)	31-128 (8)	129 (8)	40 (8)	42 (8)		38-4387 (8)	9011 (8)
(7,7-dimethyl-4-bicyclohept-3-enyl)methanol	myrtenol	6712-78-3	(7)										
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	phytol	150-86-7					66 (8)	56 (8)				15 (8)	
1-(4-methoxyphenyl)-2-methyl-3-buten-1-ol							6-16 (8)		3 (8)			16 (8)	74 (8)
1,2,3,4-butanetetrasol	erythritol	149-32-6											
1,2,3-propanetriol	glycerol, glycerine	56-81-5											
1,2-cyclohexanediol		931-17-9											

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pompona* origin unknown	*V. pompona* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9			42 (8)						11 (8)		
1,2-dimethoxybenzene	**veratrol**	91-16-7											
1,2-propandediol	propylene glycol	57-55-6											
1,3-butanediol	b-butylene glycol	107-88-0					133-2895 (8)	37 (8)	279 (8)			258 (8)	
1,3-cyclohexanediol, trans-	**hexahydroresorcinol**	504-01-8											
1,4-butanediol	tetramethylene glycol	110-63-4											
1-methoxy-2-(4-methoxyphenyl)methylbenzene													
1-octen-3-ol	octenol, vinyl hexanol	3391-86-4	(7) (9)										
1-phenyl-1,2-butanediol													
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	alpha-terpineol	98-55-5	(7)										
2,2,4-trimethyl-3-penten-1-ol													
2,2-dimethylpentan-1-ol	**neoheptanol**	2370-12-9			11 (8)		6 (8)					76 (8)	
2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	24347-58-8	(7)								3 (8)	177 (8)	
2,4-dimethyl-1-heptanol													
2,6-dimethoxy-4-methylphenol	**4-methyl syringol**	6638-05-7								90 (8)		155 (8)	
2,6-dimethyl-4-ethyl-4-heptanol													
2-acetoxy-1-propanol							6 (8)						
2-butyne-1,4-diol													
2-cis-9-octadecenyloxyethanol													
2-ethylcyclobutanol		35301-43-0			45 (8)								
2-methoxy-3-methylbutane													
2-methoxy-4-(2-propenyl)-phenol	eugenol	97-53-0	(9)										
2-methoxy-4-methylphenol	creosol	93-51-6	(7) (9)										
2-methoxy-4-prop-2-enylphenol	**engenol, caryophyllic acid**	97-53-0								12 (8)			
2-methoxy-p-cresol	**2-Hydroxy-5-methylanisole**	93-51-6			18 (8)		4-83 (8)		8 (8)	13 (8)	4 (8)	4-6 (8)	6 (8)
2-methoxyphenol	guaiacol, methylcatechol	9009-62-5	(7) (9) (16)		207 (8)	126 (8)	11-17 (8)	131 (8)		14 (8)		7-59 (8)	27 (8)
2-methyl-butan-1-ol		137-32-6	(7)										
2-octen-4-ol	butyl propenyl carbinol	20125-81-9											
2-phenylethanol	phenylethyl alcohol	60-12-8	(7) (9)										
3-(hydroxymethyl) phenol		620-24-6					45 (8)		82 (8)				
3,4-Dimethoxybenzyl alcohol	**veratryl alcohol**	93-03-8											371 (8)
3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	nerolidol	40716-66-3											
3,7-Dimethyl-2,6-octadien-1-ol	geraniol	106-24-1	(7)										
3,7-dimethyl-6-octen-1-ol	citronellol, dihydrogeraniol	106-22-9	(7)										
3-7-dimethylocta-1,6-dien-3-ol	linalyl alcohol, linalool	78-70-6	(7)										
3-methyl-2-buten-1-ol	prenol	556-82-1	(7)										
3-methyl-2-propylpentan-1-ol													
3-methyl-butan-1-ol	isopentanol	6423-06-9	(7)										
3-methylhexan-2-ol		2313-65-7										831 (8)	
3-methylpentan-1-ol		589-35-5	(7)										
3-methylphenol	**cresol**	108-39-4								34 (8)			
3-phenyl-2-propen-1-ol	cinnamyl alcohol	104-54-1											
3-phenylpropan-1-ol	benzene propanol	122-97-4											
4-(3-hydroxy-1-propenyl)-2-methoxyphenol	coniferyl alcohol	458-35-5	(12)										
4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol	498-00-0	(10) (30) (32)	(10)			11-2676 (8)		78 (8)			16 (8)	23 (8)
4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	(10) (14) (16) (32)	(10)		62 (8)	13-74 (8)					9 (8)	
4,5-octanediol		22607-10-9									22 (8)		
4-butoxy-1-butene													
4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	(7)		577 (8)					290 (8)			
4-ethenylphenol	4-vinyl phenol	2628-17-3											
4-ethenylphenol	p-vinyl phenol	2628-17-3	(7)										
4-Ethyl-1,3-benzenediol	4-ethylresorcinol	2896-60-8							20 (8)		4 (8)	7 (8)	
4-ethyl-2-methoxyphenol	p-ethyl guaiacol	2785-89-9	(7)										
4-Methoxybenzenepropanol		5406-18-8					4 (8)					103 (8)	
4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	terpinen-4-ol	562-74-3	(7)										
4-methylphenol	p-cresol	106-44-5	(7) (9) (16)										
9,12-octadecadien-1-ol	linoleyl alcohol	506-43-4											
9-octadecen-1-ol	oleyl alcohol	143-28-2											
benzene-1,4-diol	hydroquinone, quinol	123-31-9										64 (8)	
but-2-yne-1,4-diol		110-65-6											
docosan-1-ol	behenyl alcohol	661-19-8											
dodecan-1-ol	lauryl alcohol	112-53-8	(7)										
dodecylcyclohexanol													

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pompona* origin unknown	*V. pompona* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
heptacosan-1-ol		2004-39-9			180 (8)	1034 (8)	1391-1582 (8)	781 (8)		1483 (8)	1705 (8)	486-1063 (8)	203 (8)
heptadecan-1-ol	heptyl alcohol	111-70-6	(7)										
heptan-2-ol	amyl methyl carbinol	543-49-7											
hexacosan-1ol		506-52-5			36 (8)		8-299 (8)		249 (8)		164 (8)	94 (8)	88 (8)
hexadecan-1-ol	cetyl alcohol, palmityl alcohol	36653-82-4											
hexan-1-ol	hexyl alcohol	111-27-3	(7)										
hexan-2-ol		626-93-7				5 (8)	179-849 (8)		1023 (8)	22 (8)		4-18 (8)	17 (8)
hydroxytricosane													
methyl-2-vinylethyl carbinol													
nonan-2-ol		628-99-9	(7)										
nonan-3-o1		624-51-1							51 (8)				
o-catechol													
octacosan-1-ol	montanyl alcohol	557-61-9					22-1732 (8)	966 (8)	7 (8)		703 (8)	7 (8)	244 (8)
octan-1-ol	octyl alcohol, capryl alcohol	111-87-5	(7)										
pentan-1-ol		71-41-0	(7)										
pentan-2-ol	amyl alcohol	6032-29-7	(7)										
phenol	phenyl alcohol, benzenol	108-95-2	(7)		17 (8)	5 (8)	3-32 (8)	54 (8)		27 (8)		6-311 (8)	8 (8)
phenylmethanol	benzyl alcohol	100-51-6	(7)										
tetradecan-1-ol	myristyl alcohol	112-72-1											
ALDEHYDES													
(2E,4E)-deca-2,4-dienal		80-56-8							126 (8)	127 (8)			
2,2-dimethylpent-4-enal		5497-67-6											
2,6,6-trimethylcyclohexene-1-carbaldehyde	beta-cyclocitral	52844-21-0	(7)										
2-hydroxy-2-methylpropanal		20818-81-9											
2-hydroxybenzaldehyde	salicylic aldehyde	90-02-8	(7)										
2-methylbut-2-enal	2-methyl-2-butenal	6038-09-1											
2-oxopropanal	pyruvaldehyde	78-98-8											
3,3-dimethylhexanal					26 (8)								
3,4-dihydroxybenzaldehyde	**protocatechualdehyde**	139-85-5										31-148 (8)	
3,5-Dimethoxy-4-hydroxybenzaldehyde							24 (8)						
3-methoxybenzaldehyde	m-anisaldehyde	591-31-1											
3-methylbutanal	Isovaleraldehyde	590-86-3					19 (8)			123 (8)	15 (8)	48-87 (8)	
3-methylpentanal	3-methyl valeraldehyde	15877-57-3					76-181 (8)		157 (8)	98 (8)		67-321 (8)	308 (8)
4-hydroxy-3,5-dimethoxybenzaldehyde	syringic aldehyde	134-96-3				24 (8)	29-61 (8)	45 (8)	23 (8)	8 (8)	8 (8)	42 (8)	24 (8)
4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	(7)	(7)	23252 (8)	26713 (8)	12178-28593 (8)	29171 (8)	11901 (8)	1439 (8)	2218 6 (8)	9024-16630 (8)	4393 (8)
4-hydroxybenzaldehyde		123-08-0	(7) (12) (13) (18) (22) (27)		3533 (8)	1891 (8)	380-1103 (8)	1564 (8)	659 (8)	94 (8)	897 (8)	460-1265 (8)	364 (8)
4-methoxybenzaldehyde	anisaldehyde	123-11-5	(7) (9) (15)									20-51 (8)	134 (8)
acetaldehyde	ethanal	75-07-0	(15)										3 (8)
benzaldehyde		100-52-7	(7) (15) (25)										
dec-2-enal	2-decenal	3913-81-3											
decanal		112-31-2			55 (8)				75 (8)		24 (8)		12 (8)
hept-2-enal	2-heptenal	29381-66-6											
hexacosanal		26627-85-0			87 (8)	432 (8)	108-464 (8)	267 (8)	511 (8)	877 (8)	300 (8)	148-825 (8)	
hexanal	hexanaldehdye	66-25-1											
nonanal	nonanaldehyde	124-19-6			142 (8)				24 (8)			81-151 (8)	16 (8)
octacosanal		22725-64-0				2049 (8)	4-18019 (8)					13-2101 (8)	
octadec-9-enal	octadecenyl aldehyde	5090-41-5											
pentanal	valeraldehyde	110-62-3	(7)										
ALKANES													
1,3,5-trimethylcyclohexane		1839-63-0			23 (8)								
10-methyleicosane		54833-23-7											
11-decylheneicosane		55320-06-4											

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pompona* origin unknown	*V. pompona* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
2,2,4,6,6-pentamethylheptane	isododecane	31807-55-3											
2,2,4-trimethylpentane	**isooctane**	540-84-1											
2,3,3,4-tetramethylpentane		16747-38-9											
2,6,10,15-tetramethyl heptadecane									24 (8)				
2,7,10-triethyldodecane													
2-cyclohexyleicosane		4443-56-5											
3-ethyl-2-methylpentane		68333-81-3											
3-ethyl-3-methylpentane		1067-08-9											
4-ethylheptane		2216-32-2						283 (8)					
4-ethyltetradecane		55045-14-2											
6-ethyl-2-methyldecane		62108-21-8											
decane		73138-29-1	(7)										
docosane		629-97-0	(7)		34 (8)	321 (8)	8-136 (8)	79 (8)	40 (8)	569 (8)	126 (8)	15-908 (8)	
dodecane		94094-93-6	(7)										
heneicosane		629-94-7											
hentriacontane		630-04-6				746 (8)	78-4637 (8)	517 (8)	56 (8)	2215 (8)		372-452 (8)	451 (8)
heptacosane		593-49-7			123 (8)	279 (8)	49-327 (8)	226 (8)	265 (8)	331 (8)	177 (8)	60-293 (8)	66 (8)
heptadecane		629-78-7	(7)										
hexacosane		630-01-3			46 (8)	272 (8)	18-138 (8)	156 (8)	150 (8)	160 (8)	73 (8)	17-205 (8)	36 (8)
hexadecane		544-76-3	(7) (9)										
hexane		92112-69-1											
hexatricontane		630-06-8											
eicosane	eicosane	112-95-8	(7)										
nonacosane		630-03-5			364 (8)	278 (8)	79-431 (8)	236 (8)	126 (8)	933 (8)	246 (8)	43-362 (8)	120 (8)
nonane		111-84-2	(7)										
octacosane		630-02-4										8 (8)	
pentacosane		629-99-2			118 (8)	364 (8)	80-599 (8)	459 (8)	446 (8)	739 (8)	290 (8)	93-917 (8)	158 (8)
pentadecane		629-62-9	(7)										
pentatriacontane		630-07-9											
tetracosane		646-31-1			55 (8)	161 (8)	42-279 (8)	211 (8)	222 (8)	338 (8)	122 (8)	40-470 (8)	85 (8)
tetradecane		629-59-4	(7)										
tricosane		638-67-5					442 (8)	352 (8)	351 (8)			140 (8)	
tricosane		638-67-5			80 (8)	233 (8)	104-541 (8)			611 (8)	212 (8)	74-821 (8)	174 (8)
undecane		1120-21-4	(7)										
x-decane (branched)			(7)										

ALKENES

(1R,4aS,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	gamma-cadinene	39029-41-9	(7)										
(1S,4aS,8aR)-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	alpha-muurolene	10208-80-7	(7)										
1,2,3-trimethoxy-5-methylbenzene	**3,4,5-trimethoxytoluene**	6443-69-2										107 (8)	
1,2,3-trimethylbenzene		526-73-8	(7)										
1,2-dimethoxybenzene	veratrole	91-16-7	(7)										
1,3-dimethylbenzene	xylene	1330-20-7	(7)										
1-cyclohexen-1-ylethanone	**1-acetylcyclohexene**	932-66-1					15 (8)						
1-ethyl-4-methylbenzene	p-ethyl toluene	622-96-8	(7)										
1-methyl-4-(6-methylhept-5-en-2-yl)benzene	alpha-curcumene	644-30-4	(7)										
1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohexene	beta-bisabolene	495-61-4	(7)										
1-methyl-4-prop-1-en-2-ylcyclohexene	limonene	5989-27-5	(7)										
1-methyl-4-propan-2-ylbenzene	cymene	99-87-6	(7)										
1-methyl-4-propan-2-ylcyclohexa-1,3-diene	alpha-terpinene	99-86-5	(7)										
1-Tricosene		18835-32-0			20 (8)	117 (8)	39-115 (8)	74 (8)	71 (8)	168 (8)	55 (8)	29-333 (8)	62 (8)
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4			466 (8)	1325 (8)	146-1841 (8)	1057 (8)	156 (8)	1136 (8)	255 (8)	133-496 (8)	1350 (8)
3-eicosene		74685-33-9											
3-methylidene-6-propan-2-ylcyclohexene	beta-phellandrene	555-10-2	(7)										
4,7,7-trimethylbicyclo[3.1.1]hept-3-ene	alpha-pinene	80-56-8	(7)										
5-eicosene		74685-30-6											
7,7-dimethyl-4-methylidenebicyclo[3.1.1]heptane	beta-pinene	127-91-3	(7)										
7-methyl-3-methylideneocta-1,6-diene	myrcene	123-35-3	(7)										

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pompona* origin unknown	*V. pompona* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
9-tricosene		27519-02-4					46 (8)						
benzene	benzol	71-43-2	(7)										
docos-1-ene	**docosene**	1599-67-3					7-83 (8)	20 (8)	4 (8)		19 (8)		48 (8)
ethenylbenzene	styrene	100-42-5	(7)										
ethyl benzene	ethylbenzol, phenylethane	100-41-4	(7)										
heptacos-1-ene	**heptacosene**					735 (8)	15-221 (8)		73 (8)	1413 (8)	56 (8)	70-1467 (8)	
hexacos-1-ene	**hexacosene**	18835-33-1				49 (8)	13-60 (8)	42 (8)		121 (8)	13 (8)	15-129 (8)	19 (8)
hexadec-1-ene	**hexadecene**	629-73-2											
methylbenzene	toluene	108-88-3	(7)										
napthalene	naphthalin, antimite	91-20-3	(7) (17)										
non-4-ene		68526-55-6											
nonacos-1-ene					177 (8)	1052 (8)	103-811 (8)	371 (8)	539 (8)	1436 (8)	354 (8)	170-996 (8)	262 (8)
pentacos-1-ene						202 (8)	83-278 (8)	172 (8)	227 (8)	464 (8)	222 (8)	48-327 (8)	78 (8)
propylbenzene	1-phenylpropane, isocumene	103-65-1	(7)										
tricosene													
x-dodecene			(7)										
x-eicosene			(7)										
x-tetradecene			(7)										

ESTERS

(1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl) acetate	bornyl acetate	76-49-3											
(4-formyl-2-methoxyphenyl) acetate	acetovanillin	881-68-5											
(4-methoxyphenyl)methyl 4-hydroxy-3-methoxybenzoate	**anisyl vanillate**												(8)
(4-methoxyphenyl)methyl (E)-3-phenylprop-2-enoate	anisyl trans-cinnamate												
(4-methoxyphenyl)methyl (Z)-3-phenylprop-2-enoate	anisyl cis-cinnamate												
(4-methoxyphenyl)methyl 2-hydroxybenzoate	**anisyl salicylate**												(8)
(4-methoxyphenyl)methyl (3,4-dohydroxybenzoate)	**anisyl protocatechuate**												(8)
(4-methoxyphenyl)methyl acetate	anisyl acetate	104-21-2	(17)									45 (8)	27 (8)
(4-methoxyphenyl)methyl anisate	anisyl anisate												
(4-methoxyphenyl)methyl formate	anisyl formate	122-91-8											50 (8)
(4-methoxyphenyl)methyl hexadecanoate	**anisyl stearate**				117 (8)							107-204 (8)	248 (8)
(4-methoxyphenyl)methyl hexanoate	**p-anisyl hexanoate**	6624-60-8										9 (8)	
(4-methoxyphenyl)methyl hydroxybenzoate	anisyl 4-hydroxybenzoate												
(4-methoxyphenyl)methyl octadec-9-enoate	**anisyl oleate**											53-178 (8)	937 (8)
(4-methoxyphenyl)methyl octadec-9,12-dienoate	**anisyl linoleate**												(8)
(4-methoxyphenyl)methyl octadec-9,12,15-trienoate	**anisyl linolenate**												(8)
(4-methoxyphenyl)methyl octadecanoate	**anisyl petadecanoate**												18 (8)
(4-methoxyphenyl)methyl petadecanoate	**anisyl palmitate**				46 (8)							37 (8)	512 (8)
(4-methoxyphenyl)methyl propenoate	**anisyl acrylate**												
(4-methoxyphenyl)methyl tetradecanoate	**anisyl myristate**												34 (8)
(4-propan-2-ylphenyl) acetate	**4-isopropylphenyl acetate**											34 (8)	(8)
[(1R,4S,6R)-1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl] acetate	isobornyl acetate	125-12-2											
[(2S)-2,3-dihydroxypropyl] (9Z,12Z)-octadeca-9,12-dienoate	2,3-dihydroxypropyl linoleate	2277-28-3											
[(E)-3-phenylprop-2-enyl] (E)-3-phenylprop-2-enoate	cinnamyl cinnamate	122-69-0	(17)										
[(E)-3-phenylprop-2-enyl] benzoate	cinnamyl benzoate	5320-75-2	(17)										
2-(4-methylcyclohex-3-en-1-yl)propan-2-yl acetate	alpha-terpinyl acetate	80-26-2											
2,3-dihydroxypropyl acetate	**glycerolmonoacetate**	93713-40-7			480 (8)		138-711 (8)	391 (8)	408 (8)	50 (8)	149 (8)	109-125 (8)	
2-acetyloxyethyl acetate	**ethylene diacetate**	111-55-7					8-71 (8)		15 (8)			15-73 (8)	
2-ethylhexyl (E)-3-(4-methoxyphenyl)prop-2-enoate	**2-Ethylhexyl-4-methoxycinnamate**	5466-77-3					5 (8)						
2-ethyl-trans-bicyclohexyl methyl 14-methylpentadecanoate													
2-hydroxyethyl acetate	ethylene glycol acetate	65071-98-9											
2-methylpropyl pentanoate	isobutyl valerate	10588-10-0	(7)										
2-pentanal propanoate													
2-phenethyl formate		104-62-1											
2-phenylethylacetate		103-45-7	(7)										
3,7-dimethyloct-6-enyl 2-methylpropanoate	citronellyl isobutyrate	97-89-2											
3,7-dimethylocta-1,6-dien-3-yl acetate	linalyl acetate	115-95-7											
3-hydroxypropyl (Z)-octadec-9-enoate	3-hydroxypropyl oleate												

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pomponia* origin unknown	*V. pomponia* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
3-hydroxypropyl prop-2-enoate	**2-hydroxypropyl acrylate**	25584-83-2											218 (8)
3-methylbutyl 2-hydroxybenzoate	isoamyl salicylate	87-20-7											
4-hexen-1-ol acetate													
7-methyl-4-octanol acetate													
butyl hexanoate	butyl caproate	626-82-4											
butyl pentanoate	butyl valerate	591-68-4	(7)										
diethyl benzoate			(7)										
ethenyl formate	vinyl formate	692-45-5											
ethyl 2-(4-hydroxy-3-methoxyphenyl)acetate	**ethyl homovanillate**	60563-13-5					249 (8)		28 (8)			31-36 (8)	
ethyl 2-hydroxy-2-methylbutanoate	ethyl-2-methyl butyrate	77-70-3	(7)										
ethyl 2-hydroxybenzoate	ethyl salicylate	118-61-6											
ethyl 2-hydroxypropanoate	ethyl lactate	97-64-3	(7)										
ethyl 2-methoxyacetate	ethyl methoxyacetate	3938-96-3	(7)										
ethyl 4-hydroxy-3-methoxybenzoate	**ethyl vanillate**	617-05-0					192 (8)					32 (8)	
ethyl 4-oxopentanoate	ethyl levulinate	539-88-8	(7)										
ethyl acetate		141-78-6			455 (8)	74 (8)		32 (8)		50 (8)			
ethyl hexadecanoate	ethyl palmitate	628-97-7	(7)		31 (8)		8-72 (8)						
ethyl hexanoate	ethyl caproate	123-66-0	(7)										
ethyloctadeca-9,12,15-trienoate	ethyl linolenate	1191-41-9											
hexyl 2-hydroxybenzoate	hexyl salicylate	6259-76-3											
hexyl acetate	methamyl acetate	142-92-7					17-274 (8)	440 (8)	145 (8)			515 (8)	
hexyl acetate		142-92-7	(7)										
hexyl butanoate		2639-63-6											
methoxymethyl acetate		4382-76-7											
methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0											
methyl 10,13-octadecadienoate													
methyl 11-octadecenoate													
methyl 2-hydroxyacetate	methyl glycolate	96-35-5	(7)										
methyl 2-hydroxybenzoate	methyl salicylate	119-36-8	(7)										
methyl 2-hydroxypropanoate	methyl lactate	547-64-8	(7)										
methyl 2-oxopropanoate	methyl pyruvate	600-22-6										199 (8)	
methyl 2-phenylacetate		101-41-7	(7)										
methyl 3-methoxybenzoate	methyl m-anisate	5368-81-0											
methyl 4-(2-hydroxyethoxy)benzoate												30 (8)	
methyl 4-(hydroxymethyl)benzoate		6908-41-4											
methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	(7)		17 (8)	8 (8)	5-120 (8)	18 (8)	136 (8)		16 (8)	4 (8)	119 (8)
methyl 4-hydroxybenzoate		99-76-3											
methyl 4-methoxybenzoate	methyl anisate	121-98-2											
methyl acetate	Tereton	79-20-9							21 (8)			85 (8)	
methyl acrylate	**methyl prop-2-enoate**	96-33-3			131 (8)	128 (8)	58-464 (8)		269 (8)	171 (8)	5 (8)	41-346 (8)	200 (8)
methyl benzoate		93-58-3	(7)										
methyl dodecanoate	methyl laurate	111-82-0	(7)										
methyl heptadecanoate	methyl margarate	1731-92-6	(7)										
methyl heptanoate		106-73-0	(7)										
methyl hexadecanoate	methyl palmitate	112-39-0	(7) (9)			13 (8)	5-30 (8)	20 (8)		25 (8)		36 (8)	8 (8)
methyl hexanoate	methyl caproate	106-70-7	(7)										
methyl icosanoate	methyl arachidate	1120-28-1											
methyl nonanoate		1731-84-6	(7)										
methyl pentadecanoate		7132-64-1	(7)										
methyl pentanoate	methyl valerate	624-24-8	(7)										
methyl tetradecanoate	methyl myristate	124-10-7	(7)										
methyl-2-(4-hydroyphenoxy) benzoate													52 (8)
methyl-3-phenylprop-2-enoate	methyl-trans-cinnamate	1754-62-7	(7) (9) (17) (19)		18 (8)	15 (8)	20-28 (8)	51 (8)	14 (8)				
methyl-4-hydroxybenzoate							120 (8)		108 (8)		36 (8)	728 (8)	
methyl-8-methyldecanoate													
pentyl 2-hydroxybenzoate	pentyl salicylate	2050-08-0											
pentyl acetate	n-amyl acetate	628-63-7	(7)										
phenylmethyl (E)-3-phenylprop-2-enoate	benzyl cinnamate	103-41-3	(17)										
phenylmethyl acetate	benzyl acetate	140-11-4	(7)										
phenylmethyl benzoate	benzyl benzoate	120-51-4	(17)										
phenylmethyl butanoate	benzyl butyrate	103-37-7	(17)										
phenylmethyl formate	benzyl formate	104-57-4	(7)										
prop-2-enyl octadecanoate	allyl stearate	6289-31-2											

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pompona* origin unknown	*V. pompona* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
propan-2-yl acetate	isopropyl acetate	108-21-4			8 (8)								
propan-2-yl pentanoate	isopropyl valerate	18362-97-5	(7)										
propyl 4-hydroxybenzoate	**propyl paraben**	94-13-3			30 (8)								
propyl pentanoate	propyl valerate	141-06-0	(7)										
ETHERS													
1,2-dimethoxyethane	ethylene glycol dimethyl ether	110-71-4											
1-methoxyhexane	**methyl hexyl ether**	4747-07-3			36 (8)								
1-methoxypropane	**methyl propyl ether**	557-17-5											
1-propoxypropane	propyl ether	111-43-3											
2-ethoxypropane	isopropyl ethyl ether	625-54-7					20 (8)					107 (8)	
2-methoxypropane	methyl isopropyl ether	598-53-8											
3-ethenoxyprop-1-ene	vinyl allyl ether	3917-15-5											
3-methoxypentane	1-Ethylpropyl methyl ether	36839-67-5											
4-(ethoxymethyl)-2-methoxyphenol	vanillyl ethyl ether	13184-86-6	(5) (9)										
4-(ethoxymethyl)phenol	p-hydroxybenzyl ethyl ether	57726-26-8	(5) (7)										
4-(methoxy)-2-methoxyphenol	vanillyl methyl ether		(5) (27)										
anisole	methoxybenzene	100-66-3	(7)										
phenoxybenzene	diphenyl ether	101-84-8	(7)										
	anisyl ethyl ether												
	anisyl methyl ether												
	dianisyl ether												
	isopentyl methyl ether												
	methyl cyclobutyl ether						133 (8)						
	p-cresyl isopropyl ether		(7)										
	p-hydroxybenzylmethyl ether		(5) (7)										
KETONES													
(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	alpha-ionone	9066-80-2											
(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	beta-ionone	79-77-6											
(E)-oct-3-en-2-one		1669-44-9	(7)										
1-(2,4,6-trimethylphenyl)ethanone	2,4,6-trimethylacetophenone												
1-(2,4-dihydroxyphenyl)ethanone	2,4-dihydroxyacetophenone	89-84-9					3 (8)						6 (8)
1-(2-hydroxy-5-methylphenyl)ethanone	o-acetyl-p-cresol	1450-72-2				177 (8)	91-511 (8)	68 (8)	165 (8)			129-953 (8)	134 (8)
1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2				40 (8)	8-226 (8)	37 (8)	38 (8)	20 (8)	35 (8)	25-52 (8)	44 (8)
1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2503-46-0				10 (8)	31-690 (8)		58 (8)			60 (8)	
13-methyl-oxacyclotetradecane-2-11-dione													
1-hydroxyheptan-2-one			(7)										
1-hydroxypentan-2-one			(7)										
1-hydroxypropan-2-one	hydroxy acetone, pyruvic alcohol	116-09-6			349 (8)	29 (8)	114-351 (8)	436 (8)	462 (8)	114 (8)		66-397 (8)	73 (8)
1-methoxypropan-2-one	methoxyacetone	5878-19-3											
1-phenylethanone	acetophenone	98-86-2	(15)										
2,3,3,4-tetramethylcyclobutan-1-one							4 (8)						
2,4-dimethylpentan-3-one	**diisopropyl ketone**	565-80-0										57 (8)	
2-butan-2-ylcyclopentan-1-one									66 (8)			113-124 (8)	
2-hydroxy-3-methylcyclopent-2-en-1-one		80-71-7											
2-methylpentan-2-one													
3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one	**b-hydroxypropiovanillone**						16-280 (8)					27 (8)	
3-hydroxybutan-2-one	**2-acetoin**	513-86-0	(7)				147-215 (8)	21 (8)	73 (8)			28 (8)	23 (8)
3-methyl-3-decen-5-one													
3-methylbut-3-en-2-one		814-78-8											
3-methylbutan-2-one	isopropyl methyl ketone	563-80-4								65 (8)			
3-methylcyclohex-2-en-1-one		1193-18-6											

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pomponia* origin unknown	*V. pomponia* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
3-methylcyclohexan-1-one		625-96-7											
3-methylcyclopentan-1-one		6672-30-6					10 (8)						
3-methylpentan-2-one	**sec-butyl-methyl ketone**	565-61-7					9-19 (8)					50 (8)	27 (8)
3-Oxabicyclo[3.2.0]heptane-2,4-dione		7687-27-6								194 (8)	183 (8)		
3-penten-2-one	methyl propenyl ketone	625-33-2	(7)										
4-(4-hydroxy-3-methoxyphenyl)butan-2-one	zingerone,vanillyl acetone	122-48-5				19 (8)	72 (8)					58 (8)	31 (8)
4-(4-hydroxyphenyl)-2-butanone	raspberry ketone	5471-51-2											
4,4-dimethyl-2-oxethanone													
4-acetyl-2-hydroxy-5-methylbenzene													
4-butoxy-3-methylbutan-2-one							4 (8)						
4-methylene-2-oxethanone													
5-hydroxy-2,3-dimethyl-2-cyclopentene-1-one							98 (8)						
5-hydroxyheptan-2-one			(7)										
6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	502-69-2	(7)				9-28 (8)			56 (8)		21-72 (8)	
butan-2-one	methyl ethyl ketone	78-93-3											
butane-2,3-dione	diacetyl	431-03-8	(15) (27)			7 (8)	2-31 (8)		150 (8)	23 (8)		7-25 (8)	5 (8)
cycloheptanone	suberone	502-42-1							65 (8)		2 (8)	55 (8)	
cyclohexane-1,3-dione	dihydroresorcinol	504-02-9							58 (8)				
cyclohexane-1,4-dione	tetrahydroquinone	637-88-7											
cyclohexanone		108-94-1			147 (8)	103 (8)	36-201 (8)	97 (8)		125 (8)	8 (8)	61-458 (8)	195 (8)
cyclopent-4-ene-1,3-dione		930-60-9			453 (8)	51 (8)	22-50 (8)	157 (8)	137 (8)	272 (8)	5 (8)	21-318 (8)	
decan-2-one	octyl methyl ketone	693-54-9	(7)										
di(phenyl)methanone	**diphenyl ketone**	119-61-9									78 (8)		
hentriacontene-2,4-dione						511 (8)	593-1574 (8)	608 (8)	1119 (8)	1040 (8)	873 (8)	296-970 (8)	154 (8)
heptacosane-2,4-dione													
heptacosene-2,4-dione					4053 (8)	17124 (8)	8479-19721 (8)	13970 (8)	21844 (8)		22878 (8)	5387-14038 (8)	6186 (8)
heptan-2-one	amyl methyl ketone	29308-56-3	(7)										
heptan-4-one	dipropyl ketone	123-19-3											
heptane-2,4,6-trione	diacetyl acetone	626-53-9											
hex-5-en-2-ol		626-94-8											
hexan-2-one	propyl acetone	591-78-6	(7)										
hexane-2,3-dione		3848-24-6								438 (8)			249 (8)
hexane-2,4-dione		3002-24-2					22 (8)		79 (8)		2 (8)		
nonacosene-2,4-dione					846 (8)	4801 (8)	4863-24524 (8)	5822 (8)	5869 (8)	5257 (8)	6002 (8)	426-2086 (8)	2366 (8)
nonan-2-one	heptyl methyl ketone	821-55-6	(7)										
octa-4,6-dien-3-one			(7)										
octan-2-one	hexyl methyl ketone	27457-18-7	(7)										
pentacosane-2,4-dione							75-2503 (8)		110 (8)	15902 (8)	18 (8)	31 (8)	
pentacosene-2,4-dione									2107 (8)				
pentan-2-one	**ethyl acetone**	27157-48-8					39-106 (8)			89 (8)		8-257 (8)	272 (8)
pentane-2,3-dione	**acetyl propionyl**	600-14-6				19 (8)	4-26 (8)	33 (8)	30 (8)	18 (8)		20-34 (8)	584 (8)
HETEROCYCLIC													
1-(1H-pyrrol-2-yl)ethanone	2-acetyl pyrrole	1072-83-9	(7)										
1,3,7-trimethylpurine-2,6-dione	caffeine	95789-13-2											
1,3-benzodioxole-5-carbaldehyde	heliotropine, piperonal	120-57-0	(7)	(23) (26)									
1,4-dimethylpiperazine	**Lupetazine**	106-58-1											1411 (8)
1-furan-2-ylethanone	acetyl furan, 2-furl methyl ketone	80145-44-4	(7)		62 (8)	16 (8)	39-81 (8)	25 (8)				5 (8)	
1-furan-2-ylpropan-1-one	2-propionylfuran	3194-15-8											
1H-pyrrole-2,5-dione, ethyl-4-methyl													
2-(hydroxymethyl)-5-hydroxy-4H-pyran-4-one													
2,2,4,5-tetramethyl-1,3-dioxolane											45 (8)		
2,2'-bi-1,3-dioxolane													
2,3-dihydro-1-benzofuran	coumaran	496-16-2			89 (8)	26 (8)	19-111 (8)	106 (8)	104 (8)	138 (8)		96-131 (8)	
2,3-dihydro-2,5-dimethylfuran					312 (8)			14 (8)					
2,5-dimethyl furfural			(15)										
2,6,6-trimethyl-10-methylidene-1-oxaspiro[4.5]dec-8-ene	vitispirane	65416-59-3	(33)										
2,6-dimethyl-3(2H)-benzo-furanone													

IUPAC Name	Synonyms	CAS #	*V. fragrans* origin unknown	*V. pomponia* origin unknown	*V. pomponia* from Madagascar	Unidentified from Madagascar	Indian	Comoros	Ugandan	Indonesian	Hawaiian	PNG	Wild Type from Peru
2,6-dimethyl-4-pyranone													
2-butyltetrahydrofuran													
2-ethyl-1,3-dioxolane								11 (8)			99 (8)		
2-furancarboxylic acid methyl ester													
2-hydroxy-5-methyl furan			(7)										
2-pentylfuran	2-amyl furan	64079-01-2	(7)				3 (8)						
2-propylfuran		4229-91-8											
3,4-dimethylfuran-2,5-dione	**dimethylmaleic anhydride**	766-39-2			1142 (8)	533 (8)	499-975 (8)	569 (8)	707 (8)	1331 (8)	565 (8)	685-1216 (8)	
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one													
3,5-dimethyl-2,4-(3H,5H)-furandione													
3,7-dimethylpurine-2,6-dione	theobromine	83-67-0											
3H-pyran-2,6-dione	**glutaconic anhydride**				53 (8)	12 (8)	9-32 (8)	73 (8)			10 (8)	24-100 (8)	236 (8)
3-hydroxy-2-methylpyran-4-one	maltol	118-71-8			42 (8)	50 (8)	56-186 (8)	74 (8)	77 (8)	27 (8)	11 (8)	17-61 (8)	76 (8)
3-hydroxy-4,4-dimethyl-dihydro-2(3H)-furanone													
3-hydroxy-4,4-dimethyloxolan-2-one	pantolactone	79-50-5											
4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	(7)										
4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one	dihydroactinidiolide	19432-05-4	(7)										
4-hydroxy-2,5-dimethylfuran-3-one	**furaneol, strawberry furanone**	3658-77-3			259 (8)	133 (8)	20-44 (8)	218 (8)	59 (8)	217 (8)	9 (8)	28-330 (8)	15 (8)
4-methyl-2H-furan-5-one		22122-36-7							49 (8)				
5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	76330-16-0			10448 (8)	14506 (8)	737-6361 (8)	12826 (8)	4170 (8)	6220 (8)	6174 (8)	893-6980 (8)	404 (8)
5-(hydroxymethyl)-tetrahydro-2-furanol							10 (8)						
5,6-dihydro-2H-pyran-2-carboxaldehyde							25 (8)						
5-ethylfuran-2-carbaldehyde	**5-ethylfurfural**	23074-10-4			139 (8)	105 (8)	56-211 (8)	194 (8)	211 (8)			74-92 (8)	
5-hydroxy-2-(hydroxymethyl)pyran-4-one	**kojic acid**	67-99-2					17-49 (8)	30 (8)	95 (8)		14 (8)	17-82 (8)	
5-isopropyldihydro-3(2H)furandione													
5-methyl-3H-furan-2-one		591-12-8											
5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	(7)		229 (8)	147 (8)	27-485 (8)	56 (8)	68 (8)	319 (8)	12 (8)	49-106 (8)	14 (8)
5-methyl-tetrahydro-2-furan-methanol													
5-pentyloxolan-2-one	coconut aldehyde	104-61-0	(7)										
6-undecyloxan-2-one	hexadecalactone	7370-44-7											
dihydro-5-isopropyl-3(2H)-furanone													
dihydroxydihydromaltol													
furan-2,5-dione	maleic anhydride	24937-72-2				18 (8)							
furan-2-carbaldehyde	furfural	98-01-1	(7) (9) (15)		1632 (8)	1122 (8)	128-4588 (8)	1600 (8)	790 (8)		599 (8)	157-860 (8)	785 (8)
furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	(7)		469 (8)	165 (8)	92-7845 (8)	339 (8)	340 (8)	228 (8)	8 (8)	214-590 (8)	92 (8)
furan-2-ylmethyl acetate	furfuryl acetate	623-17-6	(17)										
furan-2-ylmethyl benzoate	furfuryl benzoate	34171-46-5	(17)										
furan-3-ylmethanol	**3-furanmethanol**	4412-91-3					1-12 (8)						
furfuryl hydroxy methyl ketone			(7)										
hydroxydihydromaltol					5569 (8)	2320 (8)	1411-3487 (8)	3466 (8)	2308 (8)	3489 (8)	1738 (8)	2383-3838 (8)	786 (8)
hydroxymaltol								173 (8)					
methyl 5-oxotetrahydro-2-furancarboxylate													
methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	611-13-2			10 (8)		51 (8)	25 (8)	78 (8)		67 (8)		
methyl pyridine-3-carboxylate	methyl nicotinate	93-60-7	(7)										
oxolan-2-one	gamma-butyrolactone	96-48-0	(7)		210 (8)	20 (8)	51-100 (8)	44 (8)	146 (8)	60 (8)		50-147 (8)	66 (8)
oxolan-2-ylmethyl acetate	tetrahydro-2-furfuryl acetate	637-64-9											
pyran-4-one		29943-42-8											
tetrahydro-4,4,6,6,-tetramethyl-2H-pyran-2-one													
tetrahydroxymethylfurfuryl alcohol													
thiophene	thiofuran	110-02-1	(7)										
trans-2,3-dimethyl-tetrahydro-2-furanol													

Reference Number	Author	Year
1	Adedeji *et al.*	1993a
2	Perez-Silva *et al.*	2006
3	DaCosta and Pantini	2006
4	Werkhoff and Guntert	1996
5	Galetto and Hoffman	1978
6	Hartman *et al.*	1992
7	Klimes and Lamparsky	1976
8	Lee	2006
9	Shiota and Itoga	1975
10	Prat and Subitte	1969
11	Lhugenot *et al.*	1971
12	Anwar	1963
13	Bohnsack	1965
14	Bohnsack and Seibert	1965
15	Bohnsack	1967
16	Bohnsack	1971a
17	Bohnsack	1971b
18	Chovin *et al.*	1954
19	Gnadinger	1925
20	Stoll and Prat	1960
21	Simony	1953
22	Bonnet	1968
23	Pritzer and Jungkunz	1928
24	Walbaum	1909
25	Tiermann and Haarmann	1876
26	Busse	1900
27	Kleinert	1963
28	Morison-Smith	1964
29	Cowley	1973
30	Goris	1924
31	Goris	1947
32	Chevalier *et al.*	1972
33	Schulte-Elte *et al.*	1978

(Full citations are listed in the References section)

2.3 Adulteration of Vanilla Extracts

Given the cost of producing a quality vanilla bean extract and taking into consideration the hand pollination, labor-intensive curing process, and the equipment required for extraction, it is not surprising that unscrupulous people try to increase their profit margins by adulterating the extracts with lower quality materials that are not easily perceivable. Some of the most common ways to decrease the cost of producing the extract is to use synthetic vanillin or the more potent ethyl vanillin (Purseglove, 1981).

Synthetic vanillin can be produced by using lignin or guaiacol as a starting material. Guaiacol can be obtained from several relatively inexpensive sources such as guaiac resin, wood tar or coal tar and is commonly used. Another synthetic pathway starts with eugenol which can be found in clove oil. Lignin is present in the waste sulfite liquor from paper mills and can also serve as a starting point for synthetic vanillin production (Ranadive, 1994).

Ethyl vanillin, or 3-ethoxy-4-hydroxybenzaldehyde, is 3-4 times as potent as vanillin and can be used to increase the aroma and flavor of an extract that initially lacks one or both qualities. Ethyl vanillin has a different flavor profile and is commonly used in concert with vanillin in synthetic flavors (Havkin-Frenkel, 2011).

2.4 Authenticity Determination

As instrumental analysis evolved over the last 45 years, so has the sensitivity and complexity of testing of vanilla extracts for signs of adulteration. Further, as the

technology for detecting adulteration advanced, so did the creativity of the extract manufacturers. Considering the cost of high quality vanilla extract, it is imperative to unequivocally prove the authenticity of the material and to identify any possible tampering with adulterants. There are currently three common analytical approaches to determining authenticity: (1) characteristic component ratios, although not completely accurate for all vanilla beans, (2) isotope-mass spectrometry, and (3) site-specific quantitative deuterium NMR (John, 2004). However, it is necessary to review the adulteration method development through the years to understand how the industry has arrived at the above mentioned techniques.

2.4.1 Traditional Analytical Techniques

Initially, the authenticity of vanilla extracts were based on simplistic analytical techniques such as ethanol content, vanillin content, color and lead number which was a test designed to evaluate organic acids present.

The chemistry behind the lead number, or organic acids test, was the addition of lead salts would precipitate the organic acids in the vanilla extract and could be measured volumetrically using titration techniques (Ranadive, 1994). The level of organic acids was very well characterized for authentic extracts and deviation from the accepted range would indicate adulteration. This technique advanced as the supporting instrumentation developed and has been reported in the literature utilizing paper chromatography (Fitelson, 1963), ion-exchange chromatography (Sullivan, 1960), and gas chromatography (Fitelson and Bowden, 1968). Prior to the addition of gas

chromatography as a separation technique, it could be easily faked by the addition of organic acids to the extract. The chromatographic separation and identification of the acids present made this a harder test to falsify (Purseglove, 1981).

A battery of tests developed in the 1970's by Martin can detect the addition of ethyl vanillin or synthetic vanillin by determining the vanillin content of the vanilla extract via gas chromatography and then the contents of potassium, inorganic phosphate and nitrogen by other means (Martin et al., 1975). The results of all these tests are compiled into ratios of each component versus vanillin. The ratios are evaluated against authentic extracts and deviations can predict adulteration (Ranadive, 1994).

2.4.2 Chromatography Based Analytical Techniques

Another method of screening for adulteration and vanillin content is ultra-violet analysis. Although simple UV cannot distinguish between vanillin and ethyl vanillin, it can give an indication and vanillin content. Further, when coupled with an HPLC, the UV detector can be used to identify ethyl vanillin and other phenolics by retention time and characteristic wavelength of absorption (Sinha et al., 2007; Archer, 1989). For more positive identification of eluting compounds, HPLC can be coupled with mass spectrometry (De Jager, et al., 2007).

2.4.3. Stable Isotope Ratio Analysis

The physical structure of vanillin is the same regardless of the origin, whether derived naturally from vanilla beans, or produced synthetically from lignin, eugenol or guaiacol. The ratio of carbon-13 to carbon-12 however will change based on the method

of production. Vanillin produced naturally in vanilla beans has been reported to be enriched with carbon-13 compared to synthetically produced vanillin (Bricourt, 1974; Hoffman and Salb, 1979; Culp and Noakes, 1992). Exploiting this observation, a technique called stable isotope ratio analysis mass spectrometry, or SIRA-MS, is able to detect adulteration by addition of synthetic vanillin. However, this test can be circumvented by using carbon-13 enriched vanillin in conjunction with lignin vanilla. The mixture of the two in the right ratio will appear to be a correctly carbon-13 enriched authentic vanillin from vanilla beans. This doping of synthetic vanilla was performed by replacing the methyl group of the synthetic sample with a carbon-13 methyl group. As a result, a new method was developed in which the methyl group was removed before testing with SIRA-MS (Krueger and Krueger, 1983).

2.4.4 Site-Specific Natural Isotope Fractionation - Nuclear Magnetic Resonance

A more recent technique called SNIF-NMR, or site-specific natural isotope fractionation by nuclear magnetic resonance, is able to detect the natural displacement of hydrogen atoms by deuterium atoms in the aromatic ring of vanillin (Remaud, 1997). There are four sites that can naturally exchange hydrogen for deuterium in vanillin. These are the three lone hydrogen atoms on the ring and the hydrogens that are located in the methoxy moiety. The hydrogen in the carbonyl and the alcohol do not participate. This technique can distinguish between natural vanillin produced from vanilla beans, natural vanillin as a product of fermentation, and synthetic vanillin from other processes. Each process either enriches or depletes the amount of deuterium present. Deuterium is found at higher levels in rain water around the equator, and gradually decreases as the

latitude increases in either direction. The amount of exchange that takes place is relative to the distance from the equator, or geographical location, the altitude at which the plant absorbed the rain water, the distance from the sea and the level of rainfall. Using all of these variables, the hydrogen to deuterium ratio of the four sites of vanillin can act as a geographical fingerprint. This technique is useful for adulteration detection, as well as identification of the origin of the bean.

3. Aim of research

3.1 Research Statement

The chemistry of vanilla is exceedingly complex. Attempts to identify compounds responsible for the aroma and flavor of vanilla have confirmed this statement. As analytical instrumentation and sample preparation techniques have evolved so has the depth of information in vanilla chemistry.

Recent improvements in analytical instrumentation, as well as advancements in adsorbent materials for headspace collection, will be used together to analyze and identify low concentration volatile and semi-volatile compounds in vanilla beans that contribute to its overall complexity.

3.2 Research Objective

The overall objective of this study is the use and development of emerging analytical techniques to identify novel compounds in vanilla beans and to improve upon existing methods for targeted analysis of alcoholic extracts. This will be accomplished by:

1. Assembling a an exhaustive database of existing volatile and semi-volatile compounds identified in vanilla beans of various species and geographical origins using various isolation techniques.

2. Developing a method using sub-2µm Ultra-High Pressure Liquid Chromatography columns in a legacy High Pressure Liquid Chromatograph to analyze the main phenolic compounds in an alcoholic vanilla extract, while exhibiting the increased speed and resolution of newer instrumentation.

3. Critically comparing existing headspace GC-MS techniques for volatile and semi-volatile components of vanilla beans while employing novel adsorbent phases and techniques.

4. Developing a higher resolution Direct-Thermal-Desorption Gas Chromatography – Mass Spectrometry method for the analysis of vanilla beans than previously reported in literature, resulting in the identification of compounds that were previously elusive.

5. Synthesize and fully characterize novel compounds previously discovered using DTD-GC-MS in a wild type vanilla bean that may have sensory aroma attributes. After structure is confirmed, compounds will be evaluated by GC-Olfactometry to determine odor impact.

4. Materials and Methods

4.1 Synthesis Experiments

4.1.1 Materials

The following materials were obtained from Fisher Scientific: methylene chloride (ACS certified), hydrochloric acid (ACS reagent grade), magnesium sulfate (anhydrous), sodium chloride, and sodium bicarbonate. P-methoxy benzyl alcohol was donated by International Flavors & Fragrances. P-anisoyl chloride, palimtoyl chloride and myristoyl chloride were obtained from Sigma-Aldrich Chemical Co.

The synthesis reactions were carried out in a 500mL round bottomed 3-neck flask. An over head stirrer with a paddle blade was used for agitation. The other two entryways of the flask were used for a stream of high purity nitrogen and a thermometer. During chemical additions, the thermometer was removed to gain access to the reaction flask. The reaction flask was submersed in an ice-water bath during the reaction. For post-reaction work-up wash steps, a separatory funnel was used.

4.1.2 Methods

4.1.2.1 Synthesis Procedure

The synthesis apparatus was assembled as described above. Methylene chloride (150g), p-methoxybenzyl alcohol (20g) and triethylamine (20g) were charged into the

ice-chilled roundbottom flask under a nitrogen environment. Once the temperature of the reaction reached sub-ambient, the acid chloride (30g) was added slowly over 15 minutes. The reaction was allowed to stir for an additional 3 hours before transferring the contents of the reaction flask to a separatory funnel and washing the reaction crude with 100mL of water (2x), a saturated sodium bicarbonate solution (2x) and a brine solution (2x). The washed crude product was dried over a bed of magnesium sulfate overnight. After analysis of crude by Gas Chromatograph with Flame Ionization Detection (FID), the product was roto-evaporated under vacuum at 60ºC for 30 minutes to remove residual solvent and starting materials.

4.1.2.2 GC-FID for reaction monitoring

Monitoring of crude reaction products was done by GC-FID on an Agilent 6890. The column was an Agilent Ultra-1 (p/n 19091A-115) methyl siloxane column with dimensions of 50m length, 0.32mm inner diameter and 0.52µm film thickness. An autosampler with a 10µL syringe was used to make the injection of 0.2µL. The inlet port was held at 250°C with an initial pressure of 13.04psi. The injection port was operated in split mode with a split ratio of 50:1. This head pressure gave a linear velocity of 26cm/sec in the column. The instrument was operated in constant flow mode which increased the head pressure relative to temperature throughout the run. The gas saver function was activated at 2.0 minutes into the run. The oven used a ramped program from 75°C to 315°C at 2°C/min with a 20 minute hold at the upper temperature. The FID detector was held at 250°C and the gases plumbed to it were hydrogen at 40mL/min, air at 450mL/min and nitrogen as a make-up gas at 30mL/min. A macro to calculate

retention indicies was used. This macro is based on the Van den Dool and Kratz (1963) calculation and uses a mixture of twenty-four ethyl esters for calibration. The calibration chromatogram as well as the synthesis crude chromatograms can be located in the appendix. The solution of esters used for calibration was obtained from International Flavors & Fragrances. Agilent Chemstation software was used for data acquisition and manipulation.

4.1.2.3 GC-MSD for compound identification

Confirmation of crude reaction products was done on an Agilent 6890 Gas Chromatograph with Mass Selective Detection (MSD), model 5375. The column was an Agilent DB-1MS (p/n 122-1032) methyl siloxane column with dimensions of 30m length, 0.25mm inner diameter and 0.25µm film thickness. An autosampler with a 10µL syringe was used to make the injection of 0.2µL. The inlet port was held at 250°C with an initial pressure of 8.69psi. The injection port was operated in split mode with a split ratio of 250:1. This head pressure gave a linear velocity of 36cm/sec in the column. The instrument was operated in constant flow mode which increased the head pressure relative to temperature throughout the run. The gas saver function was deactivated during this run. The oven used a ramped program from 75°C to 320°C at 5°C/min. The transfer line from the gas chromatograph to the MSD was held constant at 250°C and the quadrupole rods and source were also held constant at 150°C. The detector scanned the mass range from 20 to 400 during the acquisition. The data acquisition and manipulation was done using Agilent Chemstation software.

4.1.2.4 NMR

A 500 mHz Nuclear Magnetic Resonance instrument from Bruker was used to obtain data to confirm the structure of synthesis products.

4.1.2.5 GC-ODP

Odor evaluation of the reaction crude product was done using an Agilent 6890 Gas Chromatograph with Mass Selective Detector (MSD), model 5375 and an Olfactory Detection Port (ODP) by Gerstel. The GC program is similar to the described method in section 4.1.2.3. The effluent from the column is split between the MSD and the ODP. Additionally, the ODP has an auxiliary helium make up gas plumbed into the base to keep the linear velocity of the sample as it enters the nose cone.

Figure 4-1: Olfactory Detection Port (ODP) by Gerstel.

Source: www.gerstelus.com

4.1.2.6 Flash Purification of crude reaction product

To purify the crude product for odor evaluation, a Isolera Flash Purification system by Biotage was used. Prior to flash chromatography, the retention factors of the crude product were determined using a TLC plate with 3:1 hexanes:ethyl acetate as a mobile phase. The retention factors were entered into the Isolera system and a SNAP cartridge (Biotage) with 25g of silica was used. After fractionation, the fractions with desired product were combined and then the solved was roto-evaporated off yielding a pure, solventless sample.

4.1.2.7 Preparation of perfumery samples for evaluation

The purified synthesis products were diluted to 1% in propylene glycol. This solution was added to a perfumery blotter drop-wise and evaluated.

4.2 HPLC Method Development

4.2.1 Materials

4-hydroxy benzoic acid, 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 4-hydroxybenzladehyde, 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-hydroxy-3-ethoxybenzaldehyde (ethyl vanillin) were obtained from Sigma-Aldrich Chemical Company, and HPLC grade water came from Fisher Scientific. A stock solution (100μg/mL) was prepared including all five compounds in HPLC grade water. This stock solution was used for all experimentation.

4.2.2 Instrumentation

All experimentation was performed on an Agilent 1100 HPLC system. All original tubing was switched to 0.005" where possible: injector seat to mixing valve, mixing valve to pre-heater block, pre-heater block to column, and column to detector. The autosampler unit was equipped with a 100uL loop for injection. The standard 13μL flow cell was used, and the column effluent was detected at 280nm using a Diode Array Detector. The frequency of the detector was set at 0.3 seconds between scans. All data was acquired and manipulated using Agilent Chemstation software (Agilent, Wilmington, DE).

4.2.3 Chromatographic Methods

4.2.3.1 Restek Ultra C-18 250mm x 4.6mm x 5μm.

The part number from Restek (Bellefonte, PA) for this column is 9174575. The injection volume was 10μL, and the column was held at a constant temperature of 35ºC. The mobile phases were 1.0% acetic acid in water and acetonitrile. Gradient elution was used from 90-45% aqueous over 20 minutes with a flow rate of 1.0mL/min.

4.2.3.2 Phenomonex Luna C-18(2) 150mm x 4.6mm x 3μm..

The part number from Phenomonex (Torrance, CA) for this column is 00F-4251-EO. The injection volume was 10μL, and the column was held at a constant temperature

of 35ºC. The mobile phases were 1.0% acetic acid in water and acetonitrile. Gradient elution was used from 90-45% aqueous over 15 minutes with a flow rate of 1.0mL/min.

4.2.3.3 Phenomonex Luna C-18(2) 100mm x 4.6mm x 3μm.

The part number from Phenomonex (Torrance, CA) for this column is 00D-4251-EO. The injection volume was 10μL, and the column was held at a constant temperature of 35ºC. The mobile phases were 1.0% acetic acid in water and acetonitrile. Gradient elution was used from 90-45% aqueous over 10 minutes with a flow rate of 1.0mL/min.

4.2.3.4 Agilent ZORBAX Eclipse Plus 100mm x 4.6mm x 1.8μm.

The part number from Agilent Technologies (Wilmington, DE) for this column is 959964-902. The injection volume was 7.0μL, and the column was held at a constant temperature of 40ºC. The mobile phases were 0.1% trifluroacetic acid in water and acetonitrile. Gradient elution was used from 85-45% aqueous over 4 minutes with a flow rate of 1.25mL/min.

4.2.3.5 ES Industries Epic C-18 SD 50mm x 4.6mm x 1.8μm.

The part number from ES Industries (West Berlin, NJ) for this column is 515A91-EC18-SD. The injection volume was 5.0μL and the column was held at a constant temperature of 45ºC. The mobile phases were 0.1% trifluroacetic acid in water and acetonitrile. Gradient elution was used from 85-40% aqueous over 3 minutes with a flow rate of 1.85mL/min.

4.3 Critical comparison of headspace techniques

4.3.1 Materials

Two Bourbon (Madagascar) vanilla beans were provided by Bakto Flavors LLC and were used for the comparison of headspace techniques. One was labeled "accepted" and the other was labeled "rejected". The latter had an alcoholic, fermented off-note of unknown origin to the odor.

4.3.2 Methods

4.3.2.1 Preparation of beans for analysis

Vanilla beans were cut into 0.5-1.0 cm pieces and immediately used for SPME, Twister (HSSE) and Purge & Trap analysis.

4.3.2.2 SPME experimentation

Solid phase microextraction experiments were done using a Gerstel MPS-2 sampling unit with a Supelco 23 gauge SPME fiber (DVB/CAR/PDMS, part #57914-U) and a thermostatted agitation unit. Samples were collected onto the fiber while the vials were held constant at 40ºC for 30minutes while being agitated at a speed of 250 revolutions per minute. Prior to sample collection, the SPME fiber was baked at 250ºC for 10 minutes with a helium purge. After the sample was collected, the fiber was desorbed into an Agilent 6890 GC inlet at 250ºC for 2 minutes. The column was an Agilent Ultra-1 (p/n 19091A-115) methyl siloxane column with dimensions of 50m

length, 0.32mm inner diameter and 0.52μm film thickness. The injection port was operated in splitless mode. The instrument was operated in constant flow mode which increased the head pressure relative to temperature throughout the run and kept the average linear velocity at 36 cm/sec. The gas saver function was deactivated during this run. The oven used a ramped program from -30°C held for 4 minutes, ramped from -30ºC to 40ºC at 60°C/min, from 40ºC to 200º at 2ºC/min and then from 200ºC to 275ºC at 4ºC/min. There was no oven hold at the end of the run. The oven was cooled to -30ºC using liquid nitrogen. The transfer line from the gas chromatograph to the Micromass AutoSpec Mass Spectrometer was held constant at 250°C and the E.I. source was held constant at 150°C. The mass spectrometer scanned the mass range from 20 to 400 during the acquisition at a rate of 0.3sec per decade with a 0.3 second interscan rate. The trap current was 500uA and the detector voltage was 165V. The data acquisition and GC control was done using Waters MassLynx software. The SPME conditions were controlled using Gerstel Maestro software.

4.3.2.3 Gerstel Thermal Desorption Unit

The thermal desorption unit, or TDU, sits on top of the inlet of a gas chromatograph. Directly beneath the TDU is the cooled injection system, or CIS. Together these two pieces of equipment allow thermal desorption of a sample followed by cryo-focusing of the vaporized sample in the inlet. This coupling of TDU and CIS is required for the analysis of stir bars for HSSE, the thermal desorption of dynamic headspace traps and for the modified direct thermal desorption method. Samples in glass tubes are inserted into the top of the TDU by a specialized rail system. The sample were

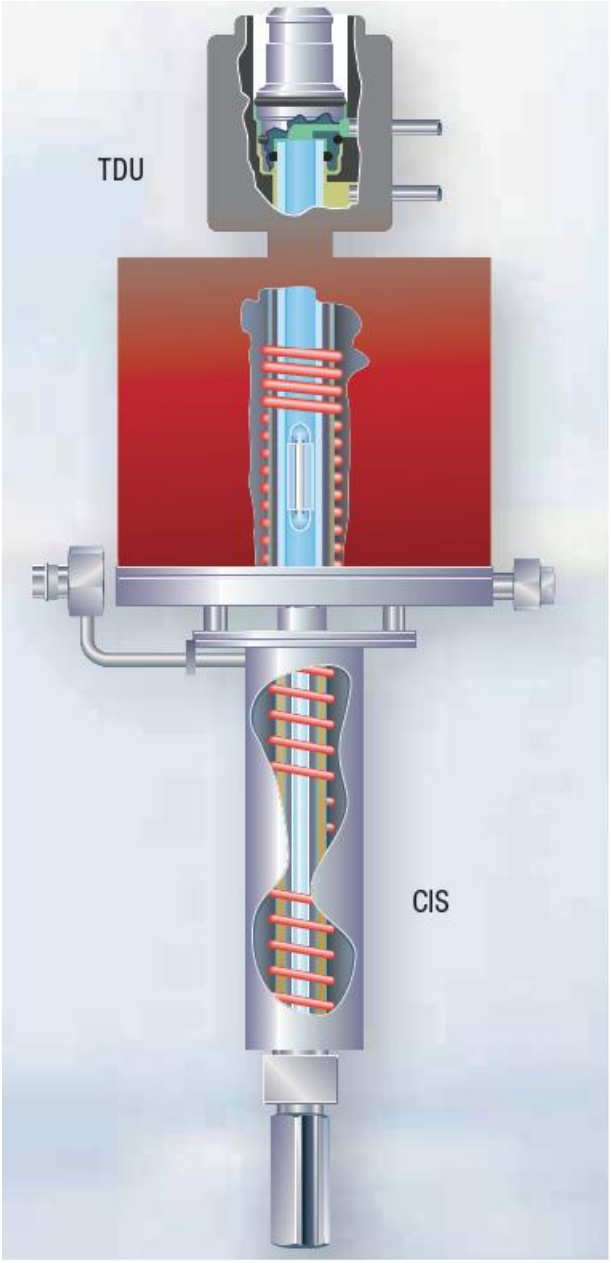

Figure 4-2: Thermal Desoprtion Unit and Cooled Inlet System by Gerstel.

Source: www.gerstelus.com

then ballistically heated to vaporize the contents of the tube. The vapor travels down the path within the TDU in a stream of helium carrier gas until it stops in the cryo-focused CIS. The CIS is cooled using liquid nitrogen to -120°C. Once the TDU tube has been desorbed for a set amount of time and all volatiles have been trapped in the CIS, the liquid nitrogen circulation is stopped and the inlet is heated at a rapid rate. This allow the sample to be introduced into the GC column as a narrow band of analytes which fosters better resolution than the traditional method of cryo cooling the GC oven to -30°C. The cooling of the oven can lead to band broadening as some analytes will travel further down the column than others before they become fixed. The analytes in the column are then heated at the rate of the oven to begin their travel through the analytical column. By cryo-focusing in the inlet, and then rapidly heating the inlet, the sample enters the analytical column all at once.

4.3.2.4 Headspace sorptive extraction (HSSE) experiment

In the HSSE experiment, one 1.0 cm PDMS stir bar was used to collect the headspace. Prior to use, the stir bar was conditioned at 290ºC for 30 minutes under a nitrogen purge. Vanilla beans were cut into 0.5-1.0cm pieces and 1.0g was placed in the bottom of a 4dram glass vial with metallic screw top cap with foil lined septa similar to Figure 4.3. A generic paper clip was used as a hanger for the stir bar using its magnetic properties. The paper clip was inserted through the septa of the vial and bent perpendicular to the vial to give the stir bar a flat edge to stick to. The stir bar was allowed to absorb the headspace at room temperature for 60 minutes.

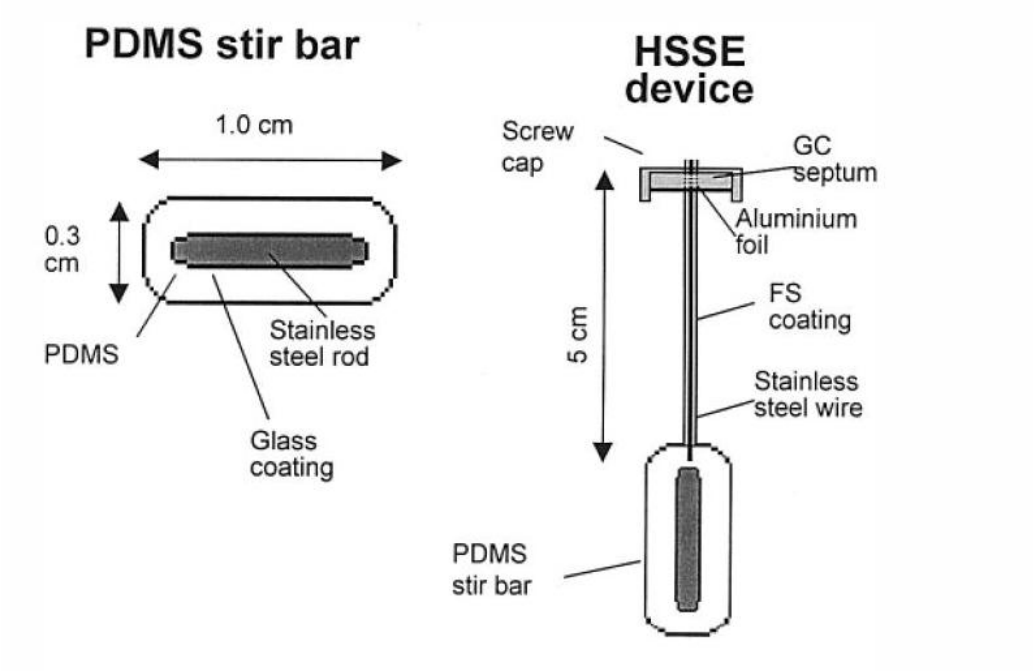

Figure 4-3: PDMS stir bar and an HSSE sampling apparatus

Source: Bicchi, et al., 2002

Immediately following the extraction process, the stir bar was placed into a blank glass TDU tube and thermally desorbed from an initial temperature of 30ºC to 275ºC at a rate of 60ºC/minute. The TDU was held at the upper temperature for 5 minutes. During the TDU heating ramp, the CIS was held at -120ºC using liquid nitrogen. Once the thermal desorption was completed and the sample was cryo-trapped at the inlet, the CIS was ramped from -120ºC to 280ºC at 12ºC/second and held at the upper temperature for 5 minutes while the GC run commenced. The thermal desorption process and the cryo-focused inlet were both held in splitless mode during this experiment. The column was an Agilent Ultra-1 (p/n 19091A-115) methyl siloxane column with dimensions of 50m length, 0.32mm inner diameter and 0.52µm film thickness. The instrument was operated

in constant flow mode which increased the head pressure relative to temperature throughout the run and kept the average linear velocity at 36 cm/sec. The gas saver function was deactivated during this run. The oven used a ramped program from 40ºC to 200º at 2ºC/min and then from 200ºC to 275ºC at 4ºC/min. There was no oven hold at the end of the run. The transfer line from the gas chromatograph to the Micromass AutoSpec Mass Spectrometer was held constant at 250°C and the E.I. source was held constant at 150°C. The mass spectrometer scanned the mass range from 20 to 400 during the acquisition at a rate of 0.3sec per decade with a 0.3 second interscan rate. The trap current was 500uA and the detector voltage was 165V. The data acquisition and GC control was done using Waters MassLynx software. The TDU and CIS conditions were controlled using Gerstel Maestro software.

4.3.2.5 Dynamic Headspace (Purge and Trap) experiment

Tubes packed with Tenax TA were used with negative pressure in conjunction with headspace sampling apparatus similar to Figure 4-4. Vanilla beans were cut into 0.5-1.0 cm pieces and 1.0g was place in the bottom of a 500ml sampling vessel. The top of the vessel contained three ports. The center port contained a charcoal filter and allowed air to enter the jar. One of the side ports contained the Tenax tube whereas the other was blocked with a sealed glass tube. Figure 4-4 shows a dual tube configuration. A single tube setup was used in this experiment. Tygon tubing connected the Tenax tube with a Gilson vacuum pump and

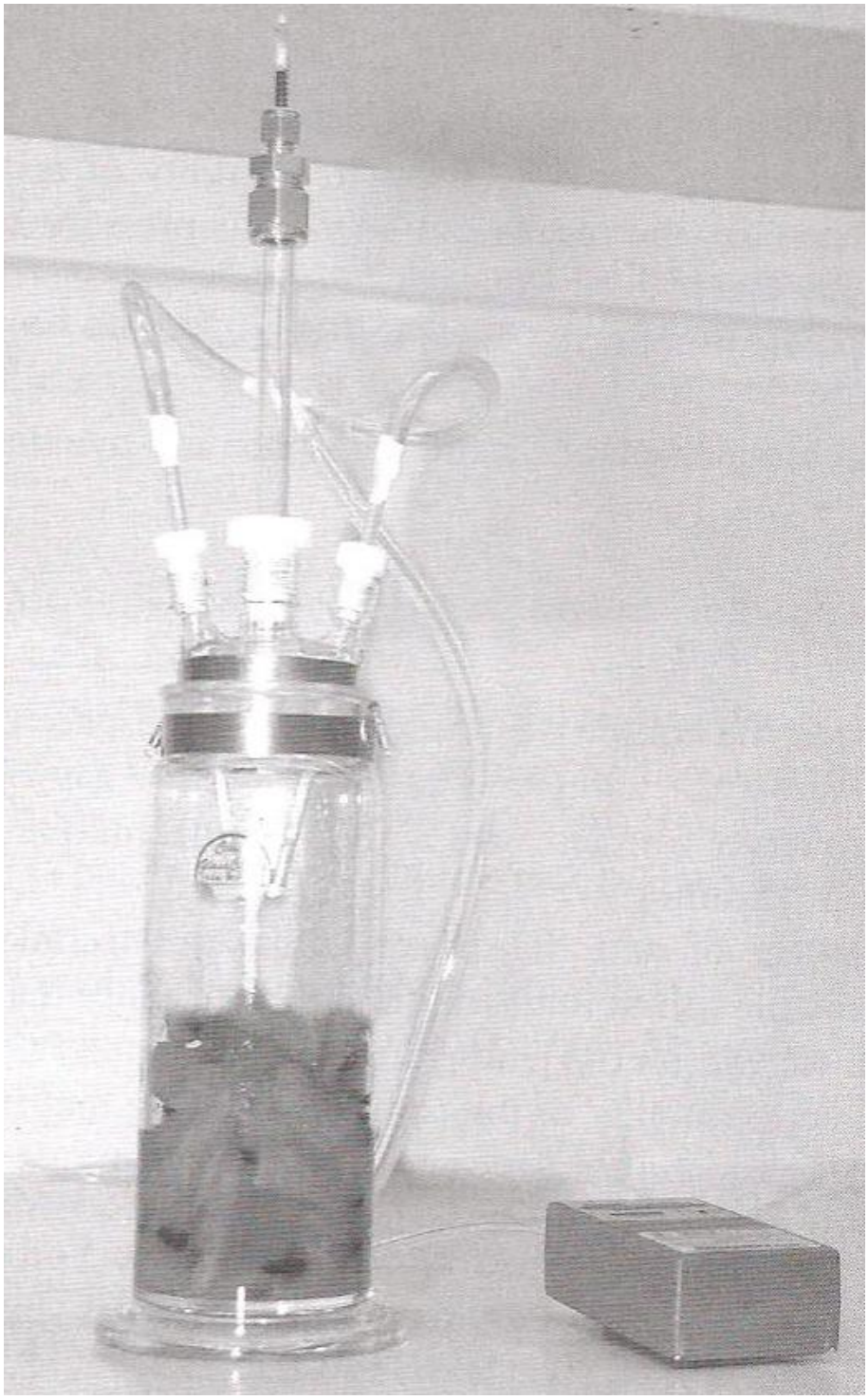

Figure 4-4: Dynamic headspace using Tenax trap and a pump.

Source: DaCosta and Eri, 2005

the headspace over the vanilla beans was collected at a rate of 50cc/minute for 20 minutes.

Immediately following the extraction process, the Tenax tube was placed into the TDU and thermally desorbed from an initial temperature of 30ºC to 275ºC at a rate of 60ºC/minute. The TDU was held at the upper temperature for 5 minutes. During the TDU heating ramp, the CIS was held at -120ºC using liquid nitrogen. Once the thermal desorption was completed and the sample was cryo-trapped at the inlet, the CIS was ramped from -120ºC to 280ºC at 12ºC/second and held at the upper temperature for 5 minutes while the GC run commenced. The thermal desorption process and the cryo-focused inlet were both held in splitless mode during this experiment. The column was an Agilent Ultra-1 (p/n 19091A-115) methyl siloxane column with dimensions of 50m length, 0.32mm inner diameter and 0.52µm film thickness. The instrument was operated in constant flow mode which increased the head pressure relative to temperature throughout the run and kept the average linear velocity at 36 cm/sec. The gas saver function was deactivated during this run. The oven used a ramped program from 40ºC to 200º at 2ºC/min and then from 200ºC to 275ºC at 4ºC/min. There was no oven hold at the end of the run. The transfer line from the gas chromatograph to the Micromass AutoSpec Mass Spectrometer was held constant at 250°C and the E.I. source was held constant at 150°C. The mass spectrometer scanned the mass range from 20 to 400 during the acquisition at a rate of 0.3sec per decade with a 0.3 second interscan rate. The trap current was 500uA and the detector voltage was 165V. The data acquisition and GC

control was done using Waters MassLynx software. The TDU and CIS conditions were controlled using Gerstel Maestro software.

4.4 TDU-GC-MS method for vanilla beans

4.4.1 Materials

Several vanilla beans were donated by International Flavors and Fragrances including Bourbon, Tahitian, Indonesian and Ugandan. Bakto Flavors LLC donated two Bourbon (Madagascar) vanilla beans labeled “accepted” and “rejected” along with a bean from Tanzania. Tenax TA absorbent, 60-80 mesh was obtained from Alltech Associates, Inc. Chromosorb W 60/80mesh and glass wool was from Sulpeco, Inc. Blank TDU tubes were obtained from Gerstel and acid washed glass wool was purchased from Restek.

4.4.2 Methods

4.4.2.1 Preparation of Solid Chromatographic Supports

Chromsorb W was used as an adsorbant aid to reduce the loss of volatile compounds during the milling process. The adsorbannt was heat treated in an oven at 200°C for 2 hours to remove any volatile contaminants. Tenax TA was used as an adsorbant bed for the addition of an internal standard. This also was heat treated in an oven at 200°C for 2 hours to remove any volatile contaminants.

4.4.2.2 Preparation of vanilla bean samples

Samples were prepared by using an IKA Analytical Grinding Mill as shown in Figure 4-5. The bottom half of the grinder was placed into a -24ºC freezer overnight before grinding. Vanilla beans were cut into 0.5-1.0cm pieces and 1.0 gram was added to the cooled grinder cup. An equivalent weight of conditioned Chromsorb W was added to the grinding cup with 2 small pieces of dry ice. The vanilla bean and Chromsorb mix was ground for 60 seconds. After the grinding procedure, the mixture was a flowable uniform powder.

Figure 4-5: IKA Analytical Grinding Mill

Source: www.ika.net

4.4.2.3 Preparation of Gerstel TDU tubes for vanilla bean samples

The blank TDU tubes contain a glass frit at the bottom of the tube. On top of this frit, a piece of glass wool was added. Above the glass wool, 100mg of conditioned Tenax TA was packed with an additional plug of glass wool above it. Using a 10uL syringe, 1.0uL of a 10mg/mL solution of 2,6-dimethoxyphenol in ethanol was added as an internal standard. The TDU tube was attached to a nitrogen supply for 10 minutes to allow the ethanol to evaporate from the Tenax TA bed. After the solvent was purged, 10mg of the vanilla bean and Chromsorb mix was added to the TDU tube followed by another plug of glass wool above it.

4.4.2.4 TDU-GC-MS method

The prepared TDU tube was placed into the TDU and thermally desorbed from an initial temperature of 30ºC to 275ºC at a rate of 60ºC/minute. The TDU was held at the upper temperature for 5 minutes. During the TDU heating ramp, the CIS was held at -120ºC using liquid nitrogen. Once the thermal desorption was completed and the sample was cryo-trapped at the inlet, the CIS was ramped from -120ºC to 280ºC at 12ºC/second and held at the upper temperature for 5 minutes while the GC run commenced. The thermal desorption process was carried out under split conditions. The split ratio was 7:1 in the TDU to prevent excessive moisture from the ground vanilla beans from freezing the inlet. The cryo-focused inlet was also held in split mode during this experiment at a ratio of 7:1. The two split ratios have an equivalent final split ratio of approximately 50:1.. The column was an Agilent Ultra-1 (p/n 19091A-115) methyl siloxane column

with dimensions of 50m length, 0.32mm inner diameter and 0.52μm film thickness. The instrument was operated in constant flow mode which increased the head pressure relative to temperature throughout the run and kept the average linear velocity at 36 cm/sec. The gas saver function was deactivated during this run. The oven used a ramped program from 40ºC to 200º at 2ºC/min and then from 200ºC to 275ºC at 4ºC/min. There was no oven hold at the end of the run. The transfer line from the gas chromatograph to the Micromass AutoSpec Mass Spectrometer was held constant at 250°C and the E.I. source was held constant at 150°C. The mass spectrometer scanned the mass range from 20 to 500 during the acquisition at a rate of 0.3sec with a 0.3 second interscan rate. The trap current was 500uA and the detector voltage was 165V. The data acquisition and GC control was done using Waters MassLynx software. The TDU and CIS conditions were controlled using Gerstel Maestro software.

4.5 Software

Spectra obtained from experiments in sections 4.3 and 4.4 were background subtracted and interpreted on a Mass-Lib data system (Max Planck Institute, Germany), using International Flavors & Fragrances in-house spectral libraries in addition to Wiley 8th, NIST 2008 and other commercial libraries.

5. Results and Discussions

The chemistry of vanilla is extremely complex. As such, no single technique can encompass all of the different attributes of vanilla aroma and flavor. Each analytical method has its own inherent advantages and disadvantages. Several techniques are described in the following sections that satisfy particular requirements of the analysis. For semi-volatile analysis, HPLC is commonly used to determine the concentrations of several key components of vanilla extracts including vanillin, 4-hydroxybenzaldehyde, vanillic acid and 4-hydroxybenzoic acid. This is a rapid screening procedure which gives useful results. However, this technique does not lend itself to new compound discovery the way that several headspace based GC-MS based techniques do. Solid phase microextraction, headspace sorptive extraction and dynamic headspace analysis are three concentrating headspace techniques for volatile and semi-volatile compounds that will be critically compared. All of these techniques are good for a range of compounds, but are especially sensitive for highly volatile compounds. Direct thermal desorption GC-MS offers an unbiased, but non-concentrated, overview of volatile and semi-volatile components. Due to the lack of bias and concentration effects, this technique works well for quantitation when coupled with an internal standard.

5.1 HPLC Method Development

5.1.1 Introduction

Many high pressure liquid chromatography (HPLC) techniques have been developed over the past two decades focusing on the detection of vanillin and other phenolic compounds from solvent extracts of vanilla beans using a variety of analytical columns to achieve better resolution and enhanced speed (Archer, 1989; Guarino and Brown, 1985; Herrmann and Stockli, 1982; Jagerdeo et al, 2000; Kahn, 1989; Lamprect et. al, 1994; Voisine et al., 1995; Wallace, 1983; Thompson and Hoffmann, 1988; Sinha et. al, 2007b). Not until very recently have scientists begun to embrace the tremendous resolving power of ultra-high pressure liquid chromatography (UPLC) and its related sub-2 micron particle size columns. Cicchetti and Chaintreau (2009) published their research on the quantitation of main compounds in vanilla extract using UPLC. However, their use of UPLC focused on increasing resolution, not speed.

It has been a recent trend in HPLC, notably evolving over the past decade, to convert methods to shorter columns with smaller diameter particles in order to achieve faster separations without sacrificing resolution or efficiency (Wu et. al, 2006; Chen and Horvath, 1995). One major limitation to using smaller particle size columns on traditional HPLC systems has been the back pressure limitation of the systems. Commercially available ultra-high performance LC (UPLC) systems that can handle the excessive pressures and related complications have been on the market for a few years. The systems are completely redesigned specifically for the higher pressures of UPLC.

The focus of this research was to use UPLC type columns (sub-2μm) in a traditional HPLC and see how fast we could make the separation before it became instrument-limited. Although traditional HPLC systems cannot handle the back pressure generated by high mobile phase flow rates, shorter columns and smaller particle size columns can be used to reduce analysis time and minimize solvent usage. Due to the recent limited availability of acetonitrile (Tullo, 2008; Swartz, 2005), solvent usage has also become a growing concern. There are several pitfalls associated with translating methods from traditional HPLC columns to sub-2μm columns, but with careful consideration, they can be successfully utilized. This research will follow the evolution of an HPLC screening method for vanillin and related phenolic compounds from its initial 250mm x 5μm C-18 column to the final method using a 50mm x 1.8μm column.

5.1.2 Chromatographic Theory

Sub-2μm diameter particles can be used to gain more resolution by increasing the number of theoretical plates per meter, or by increasing the speed of a separation using a shorter column with the same amount of theoretical plates, or by mixing the two benefits together to create a higher resolution, faster LC method (Swartz, 2005). Resolution can be expressed as an equation (Snyder et. al, 1997) with three terms: selectivity (α), efficiency (N) and retention factor (k):

$$Rs = ¼ (\alpha\text{-}1)/(\alpha) * N^{1/2} * k/(k+1)$$

The change in resolution by switching to smaller particle sizes is controlled by the efficiency term, N. Smaller particles reduce the amount of eddy diffusion in the mobile

phase as well as the amount of mass transfer resistance in the mobile phase (Wu et. al, 2006). This in turn creates more efficiency and therefore more resolution. The increased resolution allows the use of shorter columns with the same number of theoretical plates, hence, a faster separation.

The height equivalent to a theoretical plate, a measure of resolving power, is derived from several factors including particle size, linear velocity of the mobile phase, packing efficiency and diffusion characteristics. The height equivalent is related to the efficiency and column length (L) by the simplified equation:

$$H = L / N$$

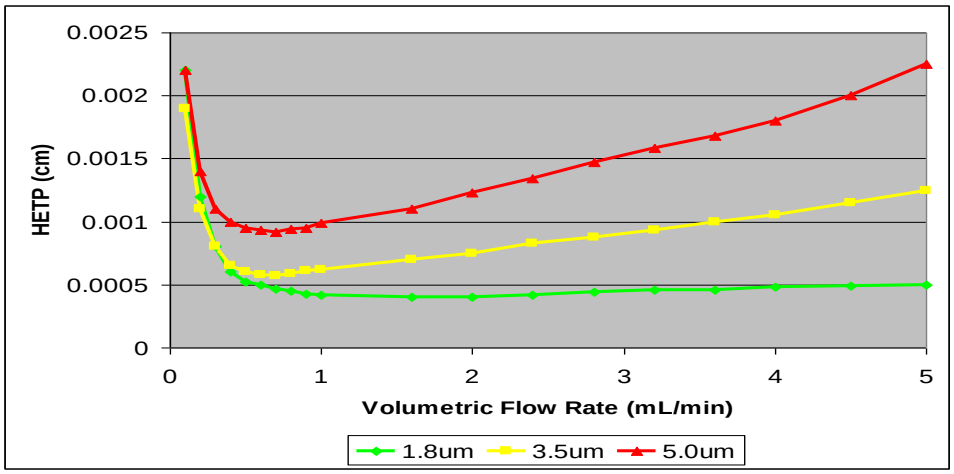

Figure 5.1-1: The van Deemter plot of Height Equivalent of Theoretical Plate (HETP) vs. volumetric flow rate of the mobile phase. Reprinted with permission from Royal Society of Chemistry Publishing.

The lower the theoretical plate height, the greater the separating power of the column. A graphical interpretation of column efficiency versus mobile phase flow rate can be seen in the van Deemter plot in Figure (5.1-1). Each particle size shown operates at maximum efficiency when the linear velocity, or volumetric flow rate, remains within the minima, or well, of the graph. This minima is the typical operating range of columns with that size packing. There is wider range of linear velocity, with preserved efficiency, for 3μm columns than there is for 5μm columns. Most importantly, the graph does not show a steep, well defined minima for the 1.8μm particle size, but rather a gently sloping line. This theoretically demonstrates that sub 2μm particle columns can operate at higher linear velocities without sacrificing resolution. Traditionally, the speed of the separation was limited by the efficiency of the stationary phase. Now it is limited only by the backpressure of the system.

5.1.3 Experimental Results and Discussion

The initial HPLC method using a 250mm x 4.6mm x 5μm C-18 column is typical of what is currently being used in laboratories for screening purposes (Sinha, 2007; Waliszewski, 2006). This column provided good resolution at the expense of speed. Ethyl vanillin, the last peak of interest, eluted at 13.45 minutes (Figure 5.1-2). Changing the column to 150mm x 4.6mm x 3μm gained efficiency by going to a smaller particle size. This added efficiency allowed a shorter column to be used. Ethyl vanillin eluted at 10.68 minutes. All of the compounds were well resolved from each other. Therefore resolution could be sacrificed to increase speed. The next experiment used a 100mm x 4.6mm x 3μm column. The only change from the previous experiment was the column

length. Ethyl vanillin now eluted at 7.02 minutes. The conversion to sub 2μm columns required several changes in the HPLC method. First, the mobile phase modifier was changed to 0.1% trifluroacetic acid to sharpen the peak shape. Second, the column temperature was increased to 40ºC to decrease the viscosity of the mobile phase, which allowed an increase in flow rate to 1.25mL/min. Under these conditions, ethyl vanillin eluted at 3.91 minutes. In this experiment, the column length and diameter were fixed, and the only change was the particle size from 3μm to 1.8μm. By definition, this increased the efficiency of the column. This added efficiency was traded off for speed in the next experiment by using a shorter column. Additional chromatograms can be found in the appendix

The last experiment in this series used a 50mm x 4.6mm x 1.8μm column. The column temperature was increased to 45ºC to control the viscosity. The flow rate was

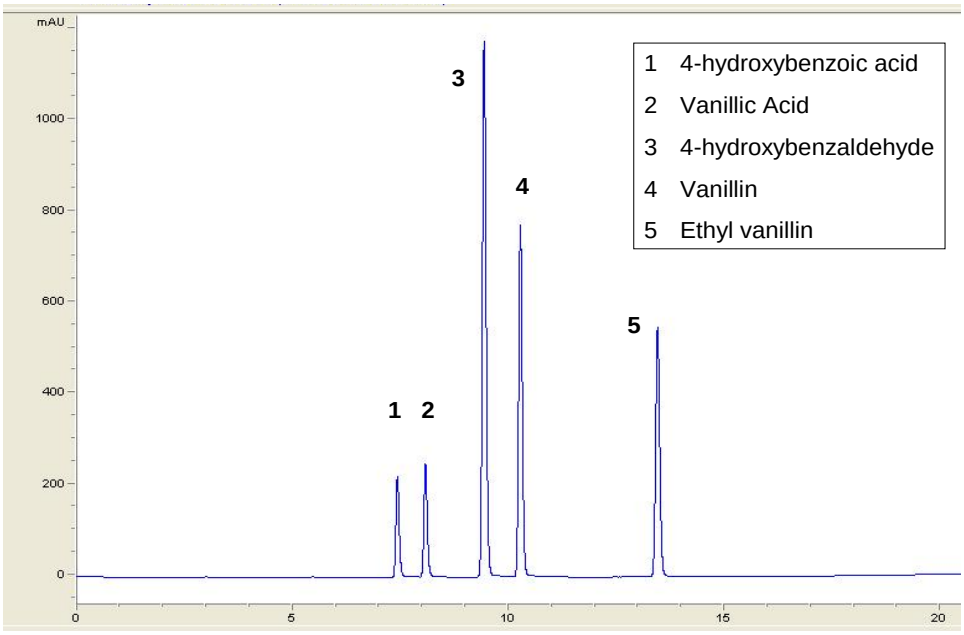

Figure 5.1-2: Chromatogram of initial method (Restek C-18 250mm x 4.6mm x 5μm. Reprinted with permission by Royal Society of Chemistry Publishing.

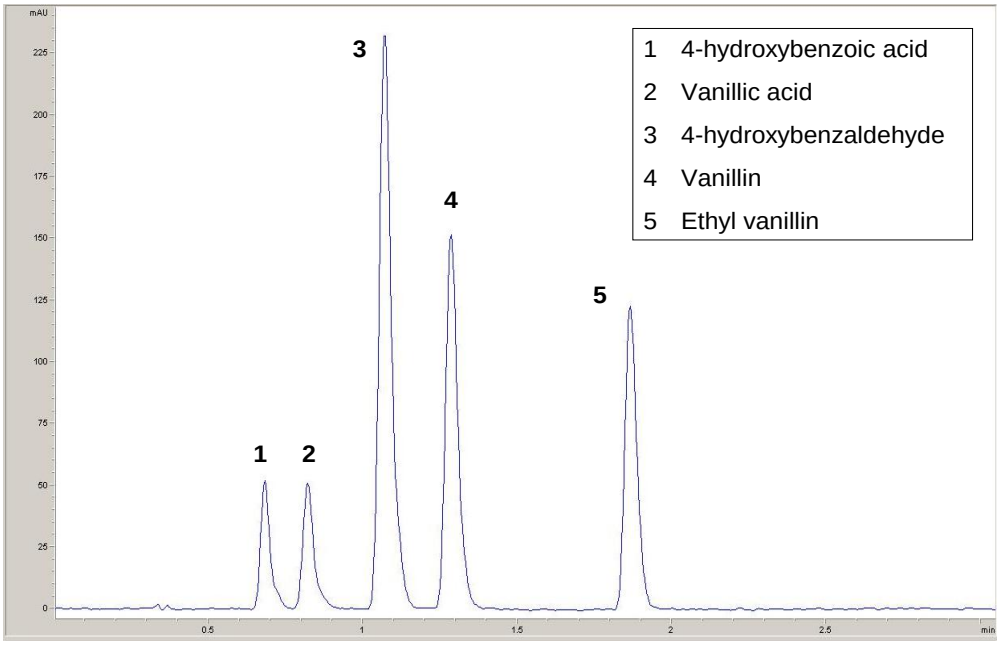

Figure 5.1-3: Chromatogram of final method (ES Industries Epic C-18 50mm x 4.6mm x 1.8μm). Reprinted with permission by Royal Society of Chemistry Publishing.

1.85mL/min and was system limited (400 bar maximum). Ethyl vanillin eluted in 1.86 minutes (Figure 5.1-3). This represents about the best that can be expected using a traditional HPLC system with a UPLC column. Although faster separations could be achieved using commercially available UPLC systems, we were still able to achieve a sevenfold increase in speed over our initial HPLC method (Figure 5.1-4). Further, the consumption of acetonitrile per 100 samples was reduced 2.7 times (Figure 5.1-5).

The plumbing of the HPLC system used for these experiments was changed from the standard 0.017" to narrow bore 0.005" in order to address two issues. First is the concern of dwell volume or system delay volume. Minimizing the volume between injection source and the entrance to the column prevents the delay of the gradient. For

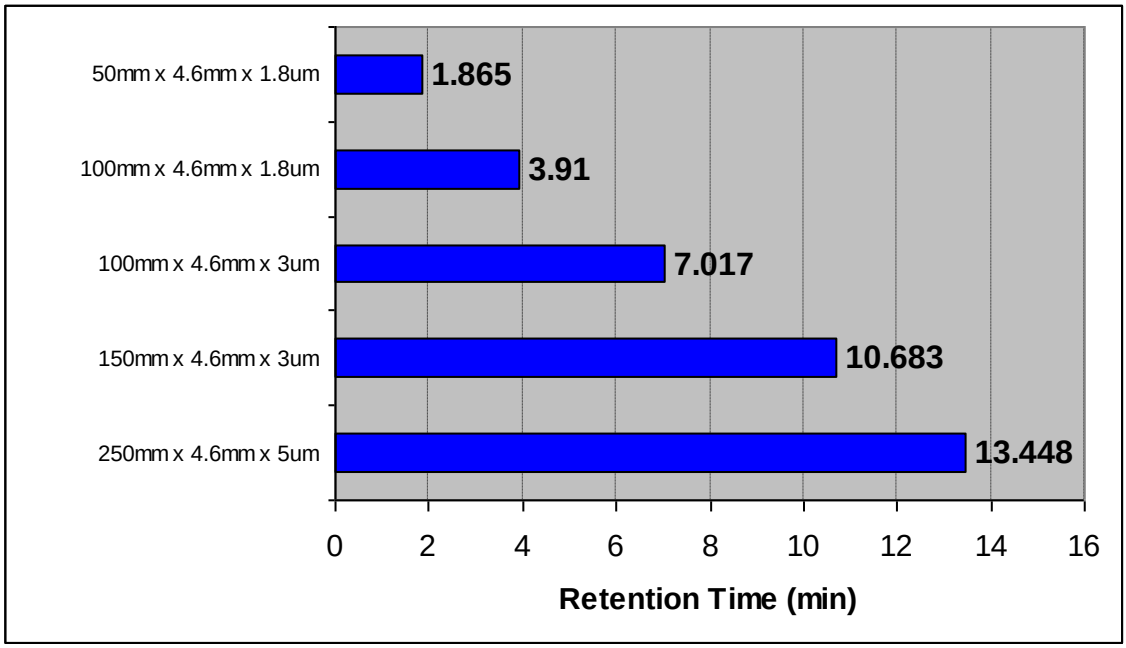

Figure 5.1-4: Retention time of ethyl vanillin vs. column length and particle size. Reprinted with permission by Royal Society of Chemistry Publishing.

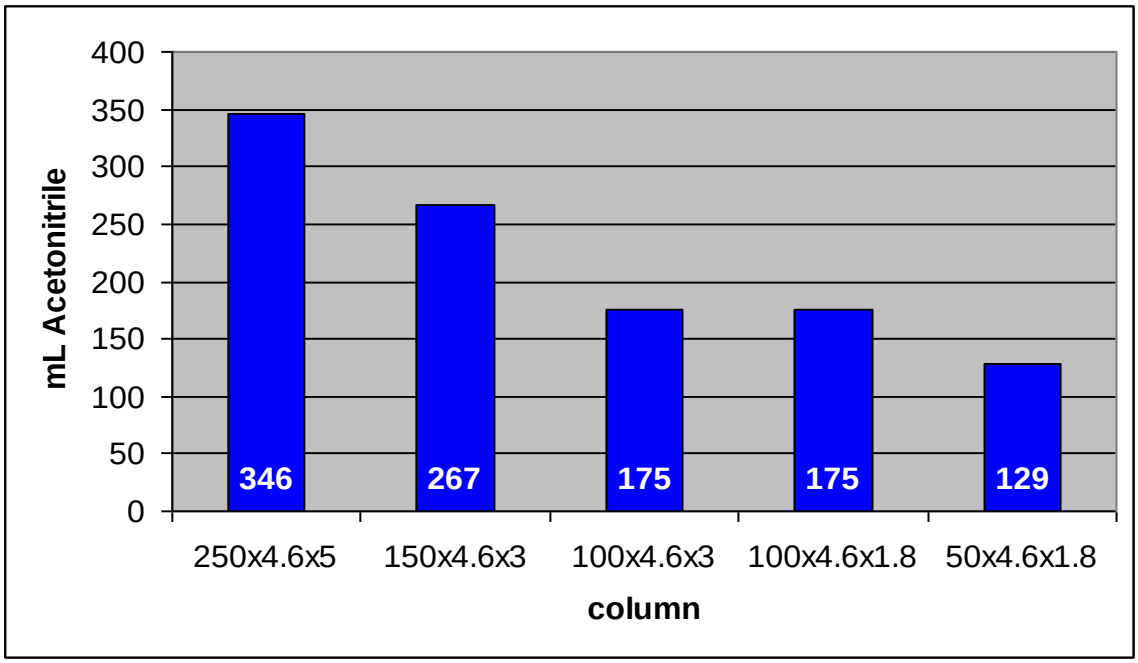

Figure 5.1-5: Acetonitrile usage per 100 samples for different columns. Reprinted with permission by Royal Society of Chemistry Publishing.

injection source and the entrance to the column prevents the delay of the gradient. For non-optimized systems, this dwell volume can cause peaks to elute isocratically, even under gradient conditions, which can cause a loss of resolution. Secondly, band broadening can occur when the ratio of column volume to non-column volume becomes skewed. When using longer columns, the volume is significantly larger than the amount of non-column volume in the system. However, when using smaller columns, the non-column volume becomes critical for maintaining resolution.

Temperature can be used to control the viscosity of the mobile phase and allow faster flow rates with reduced backpressure. Additionally, temperature of the column and mobile phase can be used to manipulate the k factor in the resolution equation and change the selectivity of the phase. The can be a benefit or a drawback depending on the separation. The pre-column heating blocks should be used to condition the mobile phase to the desired temperature. This will prevent a temperature gradient as the cooler mobile phase enters the thermostatted column at much higher temperature which could adversely affect the chromatography. Finally, the increased temperature of the mobile phase, which can be helpful in several ways, can cause unwanted baseline noise in the UV detector. Commercially available UPLC systems use a post-column cooler to remedy this.

Increased flow rate through the column and faster separations necessitate the use of a faster data capture rate. Sub 2-um particle size columns especially can create very narrow peak widths that may not be adequately collected using traditional data rates.

5.2 Volatile Analysis Technique Comparison

Recent advances in headspace sampling techniques have provided researchers with a full palette of options when analyzing samples. The methods of solid phase microextraction (SPME), headspace sorptive extraction (HSSE) and traditional dynamic headspace, or purge and trap, were critically compared when analyzing two samples of bourbon vanilla beans. Static headspace (SHS) was purposely omitted from this comparison due to the lack of sensitivity this technique provides for samples containing compounds with a wide range of volatilities. One vanilla bean sample was considered to be a good representation of what is commercially acceptable. The other was a rejected bean that had an alcoholic, fermented off-note of unknown origin to the odor. Using these three methods, the differences in aromatic composition were elucidated. Newly identified compounds are highlighted in green with bold face print in the tables below. New compounds identified in the "rejected" Bakto Flavors Bourbon vanilla bean have not been highlighted as they are not considered to be representative of, or naturally occurring in, a good quality bean.

There are several key indicators in the "rejected" Bakto Flavors bean that give clues to the origin of the off-odor. The loss of vanillin is the first key. The decreases of several key materials such as furan-2-carbaldehyde, hydroxymethylfurfural and hydroxydihydromaltol along with the presence of short chained alcohols, or fusel alcohols suggest fermentation has occurred. The depletion of vanillin and the increase in guaiacol concentration has been shown in the literature to indicate the presence of *alicyclobacillus acidoterrestris* (Bahceci, 2005). Another clue is the presence of styrene

which has been shown to be a degradation product from cinammyl compounds (Stratford, 2007). These clues support the theory that the beans were subjected to an anaerobic condition which allowed microorganisms to use vanillin as a carbon source (Havkin-Frenkel, 2012). Each headspace technique adds another piece to the puzzle and gives confirmatory clues as to the source of the off-odor.

In each of the techniques, the compounds identified are listed in table format. Compounds highlighted in yellow are considered to be outside contaminants to the experiment. Compounds highlighted in green are newly identified compounds in vanilla beans. Newly identified compounds were cross referenced with the Volatile Components in Foods (VCF) Database to identify those compounds that have been identified before in nature. Several compounds were not found in the VCF database. Although the omission of these compounds from the database indicates that they have not been previously reported, the validity of their identification in this analysis is not questioned. The VCF database is a comprehensive source of information, but is not exhaustive and new compounds are continuously added.

5.2.1 Solid phase micro extraction

Developed in 1990 by Arthur and Pawliszn, solid phase microextraction (SPME) is a relatively new sample extraction and enrichment technique (Arthur and Pawliszn, 1990). The technique employs a fused silica fiber (1-2cm in length) coated with a polymeric adsorptive material. The composition of the adsoptive bed can be tailored to polar compound, non-polar compounds or a mixed bed fiber can be used. Fibers coated

with polydimethylsiloxane (PDMS), divinylbenzene (DVB), carboxen (CBXN), polyacrylate (PA), carbowax (CW) and other phases as well as mixtures of these phases are commercially available. The fiber is extremely narrow in size and fits with the barrel of a syringe. This is an advantage and a disadvantage. The size allows the syringe containing the fiber to enter a GC inlet and directly expose the adsorptive material to allow thermal desorption of volatile compounds. The disadvantage is that due to the size restrictions of the needle, there is only a small amount of adsorbent, which provides a relatively small analyte loading capacity, and thus decreased sensitivity when compared to other more elaborate headspace techniques.

The syringe containing the fiber is inserted into an otherwise sealed vessel. The fiber is then exposed to above the sample to absorb the compounds in the headspace. This is an equilibrium based technique in that amount of solutes on the fiber coating gradually reach an equilibrium level with their surroundings which determines the maximum concentration for that compound on the fiber. To maximize the sensitivity of the analysis, the fiber must reach equilibrium with it environment. In order to reach equilibrium quicker, the sampling vessel can be heated. Because of its equilibrium dependence, SPME is considered to be a selective technique. Compounds that have a higher vapor pressure and a higher affinity for the fiber will become more concentrated and vice versa. This is an advantage over static headspace in that selected compounds will be more concentrated. However, because of its selective concentration, analysis by SPME does not give an accurate representation of true headspace above a sample.

Further, this technique is not well suited for semi-volatile compounds with a low vapor pressure.

Several studies have been published on the use of SPME fibers for the analysis volatile analytes of vanilla extracts. De Jager, *et al*., used a polyacrylate fiber for the detection and quantification of coumarin, vanillin and ethyl vanillin in vanilla extracts (De Jager, 2008). Polyacrylate fibers tend to absorb polar compounds better which was well suited for this study. Sostaric et al., also investigated volatile compounds from vanilla extracts using SPME, but used a variety of fiber types (Sostaric, 2000). They evaluated polydimethylsiloxane, polyacrylate and a mixed fiber of divinylbenzene and carbowax and concluded that for polar compounds, polyacrylate was superior. Although not ideal for their study, polydimethylsiloxane did perform better over a range of polar and non-polar compounds. The mixed bed fiber was considered to be better for polar semi-volatiles. These two research studies were both focused on targeted polar compound analysis in an alcoholic matrix. When analyzing the headspace over a broad range of polarities and volatilities, such as over vanilla beans, a mixed fiber would be advantageous. Cavalli et al., investigated the headspace composition of French olive oils using SPME and had success with using a mixed fiber consisting of divinylbenzene, carboxen, and polydimethylsiloxane for the analysis of volatile and semi-volatile compounds of varying polarities (Cavalli, 2003). Several studies have been published on the use of SPME for extraction and analysis of vanilla extracts as referenced above, but none have been reported on vanilla beans or on the use of a three phase mixed bed fiber (DVB/CBXN/PDMS) for the analysis of vanilla.

Based on the learnings from Cavalli et al., a mixed bed SPME fiber (DVB/CBXN/PDMS) was used for the analysis of the "acceptable" and "rejected" bourbon vanilla beans in this study. The beans were prepared and analyzed as described in Section 4. As expected, most of the compounds detected were very volatile. Because of the concentration effect of SPME, compounds that were present at low levels but have high vapor pressures and affinities for the polymeric adsorbents were detected. These types of compounds might have been missed by other non-concentrating headspace techniques. Interestingly, several new compounds were found in the "acceptable" bourbon vanilla bean which has not previously been reported including: 2-methylbutanoic acid, 4-hydroxybutanoic acid, heptanal, trans-2-heptenal, octanal, benzothiazole, dodecanal, and 5-methyl-2-phenylhexe-2-enal.

Figures 5.2-1 and 5.2-2 show the total ion chromatograms for the "acceptable" Bakto Flavors Bourbon vanilla bean and the "rejected" Bakto Flavors Bourbon vanilla bean. Table 5.2-1 is a list of compounds identified in the "acceptable" Bakto Flavors Bourbon vanilla bean. Table 5.2-2 lists the compounds identified in the "rejected" Bakto Flavors Bourbon vanilla bean. Both of these analyses were qualitative as there was no internal standard used and amounts listed are based solely on percent area of the integrated chromatogram.

A total of 35 compounds were identified from this analysis including several known contaminants from the packaging. These compounds: diethyl phthalate,

diisobutyl phthalate and dibutyl phthalate are highlighted in yellow in Table 5.2-1. Most of the major compounds identified in the "acceptable" Bakto Flavors Bourbon vanilla bean such as acetic acid, 3-hydroxybutan-2-one, 2,3-butanediol, 1,3-butanediol, furan-2-carbaldehyde (furfural), 2-methoxyphenol (guaiacol) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) are well known characteristic compounds of Bourbon vanilla beans (Klimes & Lamparsky, 1976; Hartman et al., 1992; Adedeji, 1993; Lee, 2006).

A total of 8 compounds were identified in the analysis of the "acceptable" Bakto Flavors Bourbon (Madagascar) vanilla bean for the first time. The newly identified compounds are 2-methylbutanoic acid, 4-hydroxybutanoic acid, heptanal, (E)-hept-2-enal, octanal, benzothiazole, dodecanal, and 5-methyl-2-phenylhex-2-enal (cocoa hexenal). Table 5.2-1a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

Table 5.2-2 lists the compounds identified in the "rejected" Bakto Flavors Bourbon vanilla bean. There are extremely high levels of 2,3-butanediol and (2-methoxy-4-methylphenol (creosol). Interestingly, 4-hydroxy-3-methoxybenzaldehyde (vanillin) was detected in this analysis at a very low level compared to the "acceptable" Bakto Flavors Bourbon vanilla bean. There are other compounds present, or absent, that give insight to the origin of the off-odor. The presence the fusel alcohol 3-methylbutan-1-ol along with the loss of furan-2-carbaldehyde indicates that fermentation has occurred.

Figure 5.2-1: Total Ion Chromatogram of "acceptable" Bakto Flavors Bourbon vanilla bean by SPME-GC-MS

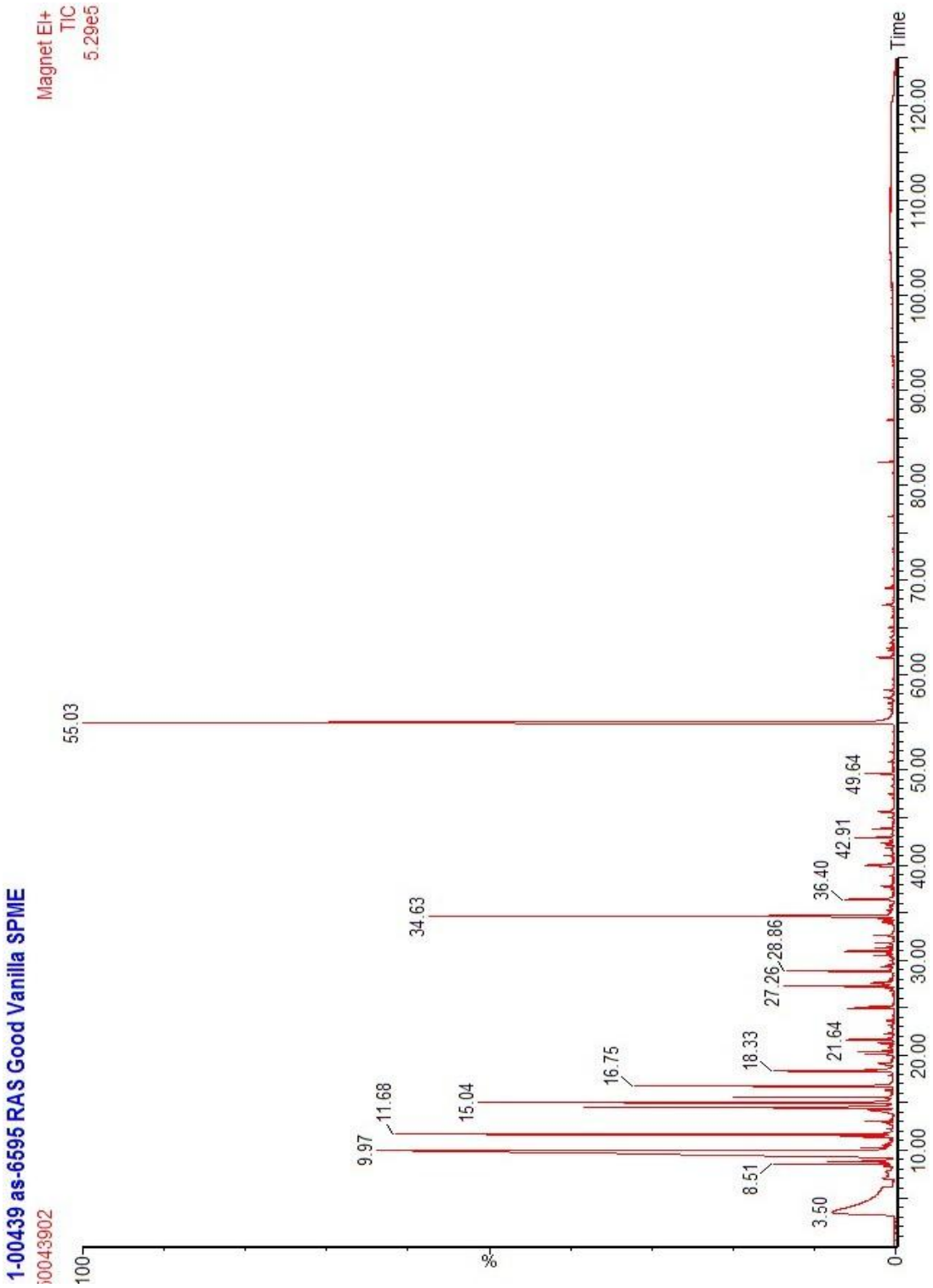

Table 5.2-1: Volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by SPME-GC-MS.

Time	Assignment	Common name	Registry #	area %
9.91	acetic acid	ethanoic acid	64-19-7	31.19
11.68	3-hydroxybutan-2-one	2-acetoin	513-86-0	4.65
14.18	2-methylpropanoic acid	isobutyric acid	79-31-2	0.59
14.53	2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	24347-58-8	4.00
14.54	methylbenzene	toluene	108-88-3	0.75
15.02	1,3-butanediol	b-butylene glycol	107-88-0	6.45
15.58	hexanal	hexanaldehdye	66-25-1	1.42
16.76	furan-2-carbaldehyde	furfural	98-01-1	3.32
18.50	3-methylbutanoic acid	isovaleric acid	503-74-2	0.50
19.10	**2-methylbutanoic acid**	**methyl ethyl acetic acid**	**116-53-0**	0.37
20.10	**4-hydroxybutanoic acid**	**gamma-hydroxybutric acid**	**591-81-1**	0.45
20.39	cyclohexanone		108-94-1	0.40
21.26	ethenylbenzene	styrene	100-42-5	0.19
21.64	**heptanal**	**aldehyde C-7**	**111-71-7**	0.55
24.96	benzaldehyde		100-52-7	0.62
25.14	**(E)-hept-2-enal**	**trans-2-heptenal**	**18829-55-5**	0.32
27.27	phenol	phenyl alcohol, benzenol	108-95-2	1.27
28.86	**octanal**	**capryl aldehyde**	**124-13-0**	1.38
34.63	2-methoxyphenol	guaiacol	9009-62-5	7.15
40.00	phenylmethyl acetate	benzyl acetate	140-11-4	0.65
42.32	2-methoxy-4-methylphenol	creosol	93-51-6	0.19
43.83	**benzothiazole**		**95-16-9**	0.33
45.00	dodecane		94094-93-6	0.09
45.62	4-methoxybenzaldehyde	anisaldehyde	123-11-5	0.25
49.64	2,6,6-trimethyl-10-methylidene-1-oxaspiro[4.5]dec-8-ene	vitispirane	65416-59-3	0.46
55.00	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	17.87
56.43	phenoxybenzene	diphenyl ether	101-84-8	0.07
57.61	**dodecanal**	**lauraldehyde**	**112-54-9**	0.13
58.42	tetradecane		629-59-4	0.14
61.86	**5-methyl-2-phenylhex-2-enal**	**cocoa hexenal**	**21834-92-4**	0.29
67.41	diethyl phthalate		84-66-2	0.21
69.40	di(phenyl)methanone	diphenyl ketone, benzophenone	119-61-9	0.04
82.43	diisobutyl phthalate		84-69-5	0.22
86.85	dibutyl phthalate		84-74-2	0.12

Table 5.2-1a: Newly identified volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by SPME-GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
2-methylbutanoic acid	blueberries (Hirvi, 1983) cranberry (Croteau, 1968) papaya (Idstein, 1985)
4-hydroxybutanoic acid	Not in VCF
heptanal	apple (Mattheis, 1991) lemon (Staroscik, 1982) guava (Idstein, 1985)
(E)-hept-2-enal	chinese quince (Mihara, 1987) olives (Flath 1973) tomato (Buttery, 1987)
octanal	apple (Mattheis, 1991) lemon (Staroscik, 1982) papaya (Flath, 1990)
benzothiazole	guava (Idstein, 1985) mango (Engel, 1983) peach (Horvat, 1990)
dodecanal	carrot (Buttery, 1968) lemon (Staroscik, 1982) orange (Coleman, 1971)
5-methyl-2-phenylhex-2-enal	roasted cocoa beans (Van Praag, 1968) roasted peanuts (Waller, 1971) roasted sesame seeds (Manley, 1974)

Figure 5.2-2: Total Ion Chromatogram of "rejected" Bakto Flavors Bourbon vanilla bean by SPME-GC-MS

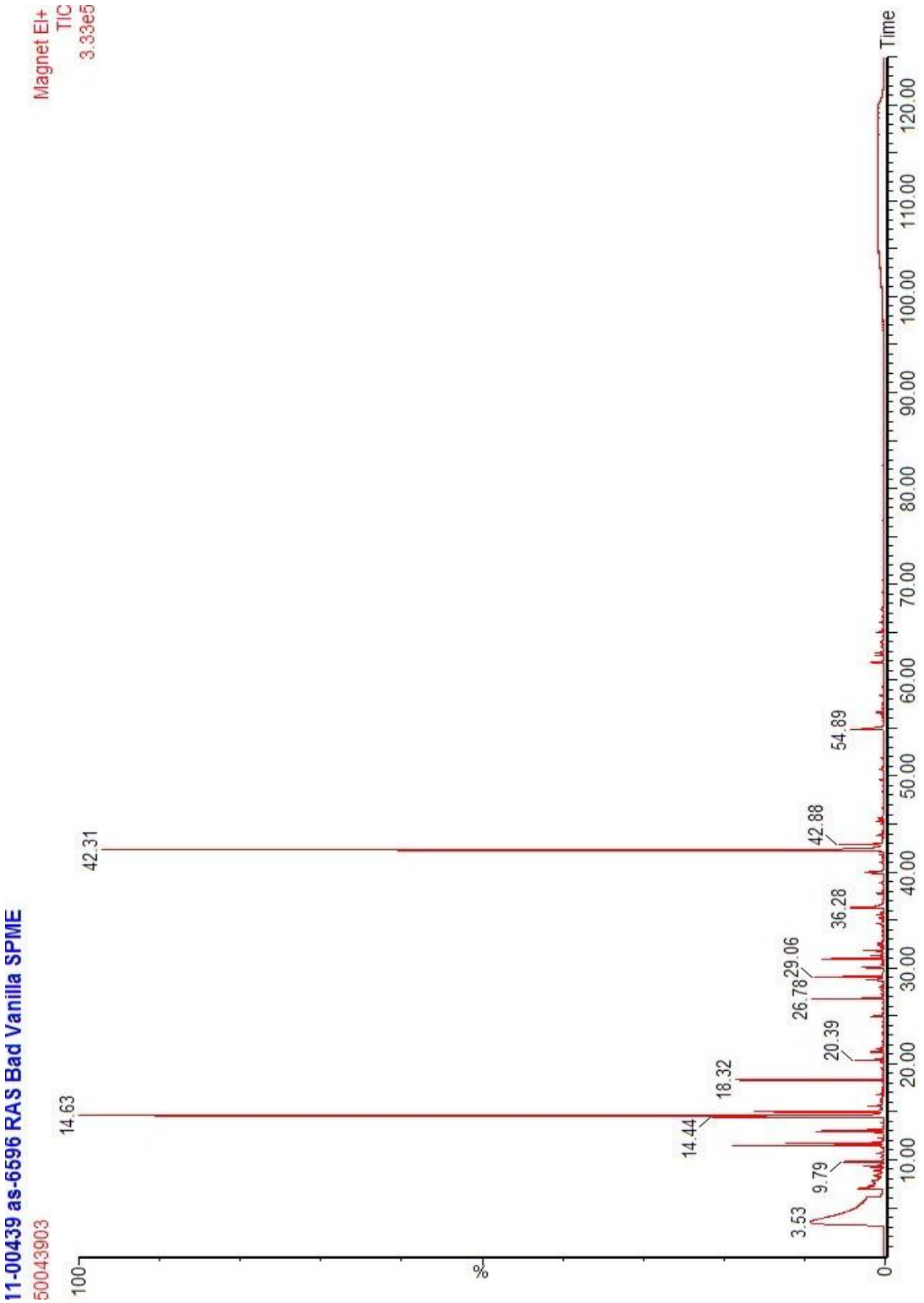

Table 5.2-2: Volatile and semi-volatile compounds identified in "rejected" Bakto Flavors Bourbon vanilla bean by SPME-GC-MS.

Time	Assignment	Common Name	Registry #	% Area
11.69	3-hydroxybutan-2-one	2-acetoin	513-86-0	1.38
12.98	3-methylbutan-1-ol	isoamyl alcohol	123-51-3	1.29
14.60	2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	24347-58-8	38.04
14.60	methylbenzene	Toluene	108-88-3	
15.00	1,3-butanediol	b-butylene glycol	107-88-0	2.47
20.39	cyclohexanone		108-94-1	0.68
21.25	ethenylbenzene	Styrene	100-42-5	0.35
24.96	benzaldehyde		100-52-7	0.31
36.29	2-phenylethanol	phenylethyl alcohol	60-12-8	1.45
42.32	2-methoxy-4-methylphenol	Creosol	93-51-6	25.02
54.89	4-hydroxy-3-methoxybenzaldehyde	Vanillin	121-33-5	1.69
67.39	diethyl phthalate		84-66-2	0.74

5.2.2 Headspace sorptive extraction

The sampling technique of headspace sorptive extraction (HSSE) is a derivative of stir-bar sorptive extraction (SBSE). Both techniques employ the same sampling apparatus, but HSSE is used in the gas phase, whereas SBSE is used on liquids. Similar to SPME, the sorbent polydimethylsiloxane is coated onto a glass encapsulated magnetic stir bar. This allows for direct immersion into the sample for SBSE and was first reported by Baltussen et al. in 1999 (Baltussen *et al.*, 1999). After the extraction has been completed, the stir bar is thermally desorbed into a GC inlet and analyzed. HSSE is a similar technique is which the coated stir bar is suspended above a sample. This was first reported by Bicchi et al. in 2000 (Bicchi *et al.*, 2000). Few studies have been published on the HSSE technique but none on the analysis of vanilla bean have been reported.

A major advantage of HSSE is the amount of sorbent present. The stir bar can be coated with up to 125µL of PDMS which give this technique superior loading capacity over SPME which uses approximately 0.5µL (Turner, 2006). Similar to solid phase microextraction, HSSE is also an equilibrium based extraction and because of its equilibrium dependence, it is also considered to be a selective technique. Compounds that have a higher vapor pressure and a higher affinity for the sorbent phase will become more concentrated and vice versa. The additional loading capacity of the phase allows further enrichment of low level compounds before equilibrium is reached making detailed analysis of trace components possible. There are a few disadvantages to the HSSE technique. Currently, polydimethylsiloxane is the only commercially available

phase. Although this is a good general purpose sorbent, it discriminated against polar compounds. Unlike SPME, mixed sorbents are not available in HSSE. Additionally, desorption requires the use of an elaborate thermal system such as the Thermal Desorption Unit (TDU) from Gerstel GmbH & Co. (Mulheim an der Ruhr, Germany) which was described with great detail in section 4. The coated stir bars are also available from Gerstel GmbH & Co. under the trademark name Twister.

Given the fact that HSSE can concentrate trace compounds, but uses a general purpose non-polar sorbent, it is not surprising that compounds detected from the two vanilla bean samples using this technique were generally present at low levels and are relatively non-polar. The thermal desorption process of the stir bars yields many artifact peaks in the chromatogram. One such peak is squalene. Although previously reported to exist in vanilla beans naturally, squalene is also known as a desorption artifact and can be detected in blank stir bar analyses. Therefore, squalene is listed as a detected compound but the relative amount is upwardly skewed.

Figures 5.2-3 and 5.2-4 show the total ion chromatograms for the “acceptable” Bakto Flavors Bourbon vanilla bean and the “rejected” Bakto Flavors Bourbon vanilla bean. Table 5.2-3 is a list of compounds identified in the “acceptable” Bakto Flavors Bourbon vanilla bean. Table 5.2-4 lists the compounds identified in the “rejected” Bakto Flavors Bourbon vanilla bean. Both of these analyses were qualitative as there was no internal standard used and amounts listed are based solely on percent area of the integrated chromatogram.

A total of 19 compounds were identified in the “acceptable” Bakto Flavors Bourbon vanilla bean. The packaging contaminants identified in the SPME-GC-MS analysis were not found in the HSSE-GC-MS analysis possibly due to the limitation of a single adsorbent versus a mixed bed fiber. 1-methyl-4-(1-methylethenyl)-cyclohexene (limonene) was present in both “acceptable” and “rejected” Bakto Flavors Bourbon vanilla bean samples. This is not considered to be a newly identified compound in vanilla beans. Rather it is an artifact in the air during the exposure/concentration step in HSSE. Limonene is ubiquitous in a fragrance research environment such as International Flavors and Fragrances, where the analysis was performed and is observed in the background of many headspace techniques, especially those that concentrate the analytes. Other compounds that have been noted as contaminants in this analysis are: hexadecanoic acid, isopropyl palmitate, 1-octadecanol and squalene. Although these are not typical packaging migrants, they are curiously present in a non-heated headspace experiment. The vapor pressures of these compounds would all but eliminate them as being authentic to the vanilla bean in this type of headspace collection. Squalene has been previously identified as a component of vanilla beans however in this analysis, the origin is most likely contamination from finger prints on the stir bar.

Four new compounds were identified in the HSSE-GC-MS analysis that have not been previously reported in vanilla beans including propyl acetate, heptanal, octanal, and (E)-3-hexen-1-ol acetate.

Figure 5.2-3: Total Ion Chromatogram of "acceptable" Bakto Flavors Bourbon vanilla bean by HSSE-GC-MS

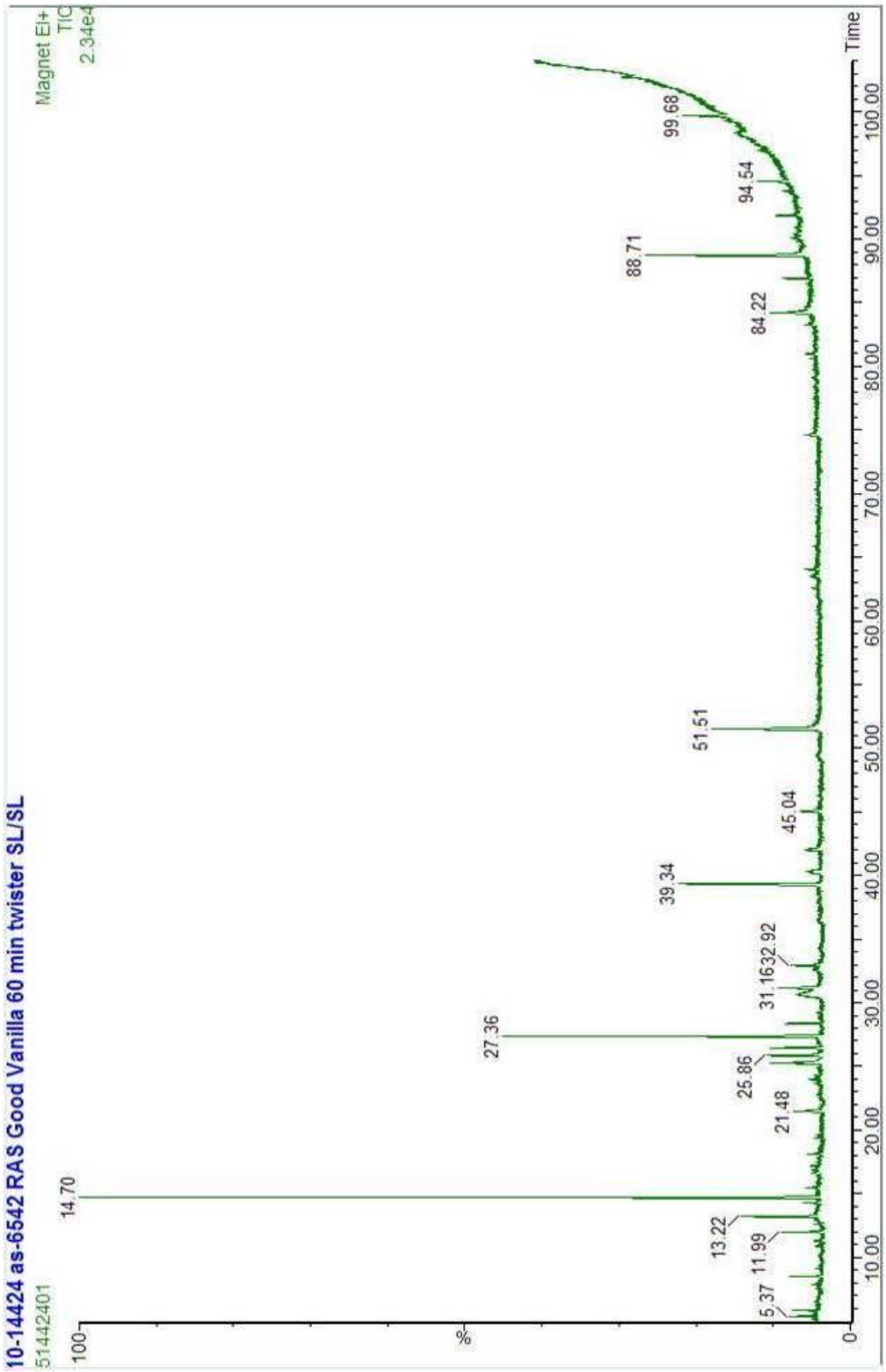

Table 5.2-3: Volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by HSSE -GC-MS.

Time	Assignment	Common Name	Registry #	% area
5.10	acetic acid	ethanoic acid	64-19-7	trace
5.80	ethyl acetate		141-78-6	trace
8.50	**propyl acetate**	**propyl ethanoate**	**109-60-4**	0.12
11.99	hexanal	hexanaldehdye	66-25-1	0.15
13.21	furan-2-carbaldehyde	furfural	98-01-1	0.41
18.02	**heptanal**	**aldehyde C-7**	**111-71-7**	trace
21.47	benzaldehyde		100-52-7	0.17
25.26	2-pentylfuran	2-amyl furan	64079-01-2	0.29
25.35	**octanal**	**capryl aldehyde**	**124-13-0**	0.08
25.85	**(E)-3-hexen-1-ol acetate**		**3681-82-1**	0.22
26.45	hexyl acetate	methamyl acetate	142-92-7	0.26
30.64	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	0.42
31.16	2-methoxyphenol	guaiacol	9009-62-5	0.26
33.00	nonanal	nonanaldehyde	124-19-6	trace
51.51	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	0.63
84.22	hexadecanoic acid	palmitic acid	57-10-3	0.21
87.00	isopropyl palmitate	palmitic acid: isopropyl ester	142-91-6	trace
88.71	1-octadecanol	stearyl alcohol	112-92-5	0.77
105.48	squalene		111-02-4	29.63

Table 5.2-3a: Newly identified volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon bean by HSSE-GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
propyl acetate	grapes (Stevens, 1969) guava (Idstein, 1985) strawberry (Dirinck, 1981)
heptanal	apple (Mattheis, 1991) lemon (Staroscik, 1982) guava (Idstein, 1985)
octanal	apple (Mattheis, 1991) lemon (Staroscik, 1982) papaya (Flath, 1990)
(E)-3-hexen-1-ol acetate	apple (Young, 1996) banana (Berger, 1986) mango (Engel, 1983)

Figure 5.2-4: Total Ion Chromatogram of "rejected" Bakto Flavors Bourbon vanilla bean by HSSE-GC-MS

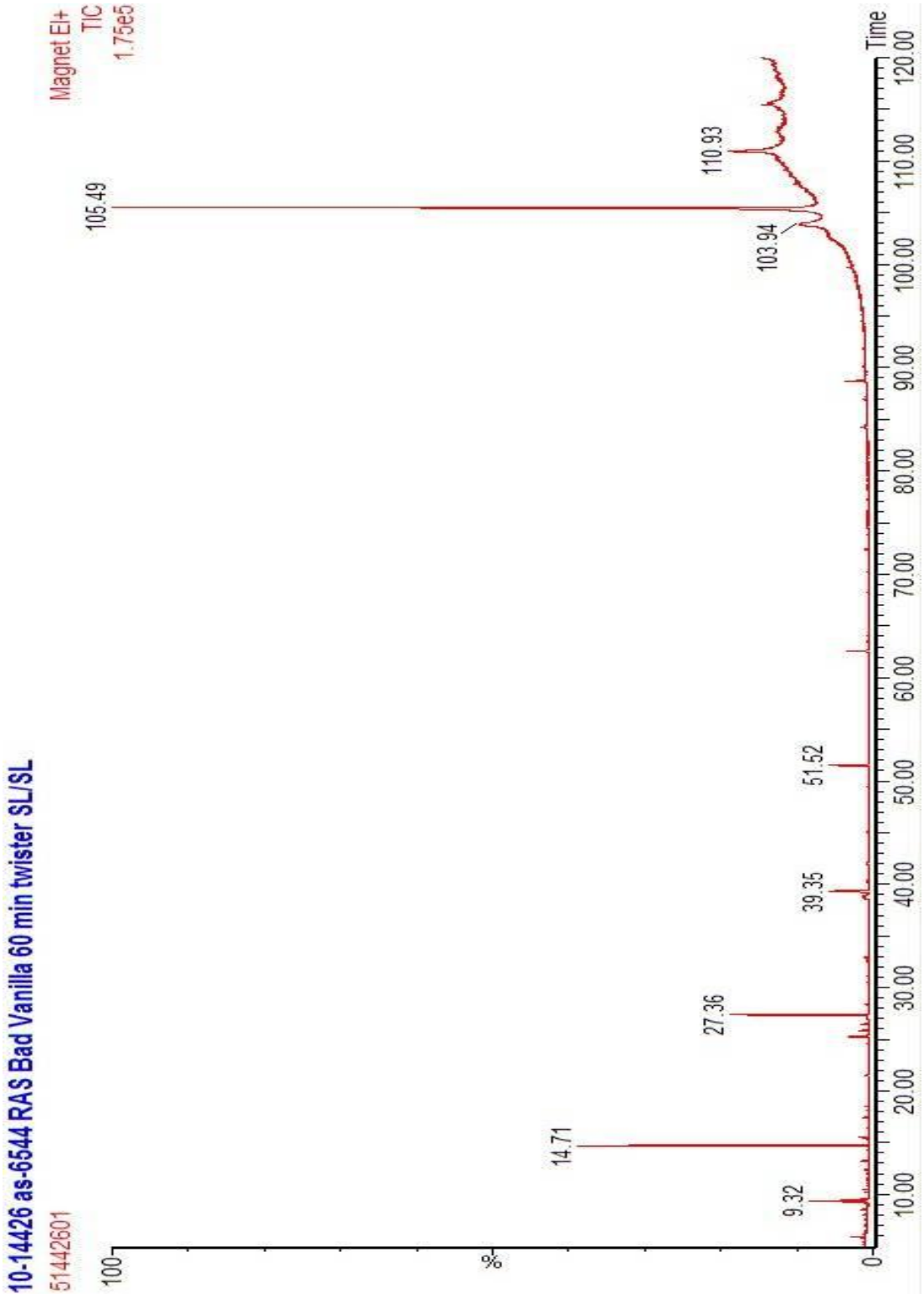

Table 5.2-4: Volatile and semi-volatile compounds identified in "rejected" Bakto Flavors Bourbon vanilla bean by HSSE-GC-MS

Time	Assignment	Common Name	Registry #	% area
5.84	ethyl acetate		141-78-6	0.26
8.51	propyl acetate	propyl ethanoate	109-60-4	0.20
9.17	2,4,5-trimethyl-1,3-dioxolane		3299-32-9	0.14
9.32	3-methylbutan-1-ol	isoamyl alcohol	123-51-3	1.77
9.49	2-methyl-butan-1-ol		137-32-6	0.81
10.47	ethyl 2-methylpropanoate	ethyl isobutyrate	97-62-1	0.17
13.23	octane		111-65-9	0.19
15.50	ethyl 3-methylbutanoate	ethyl isovalerate	108-64-5	0.25
17.43	heptan-2-one	amyl methyl ketone	29308-56-3	0.21
18.50	heptan-2-ol	amyl methyl carbinol	543-49-7	0.14
21.47	benzaldehyde		100-52-7	0.20
25.26	2-pentylfuran	2-amyl furan	64079-01-2	0.83
25.86	(E)-3-hexen-1-ol acetate		3681-82-1	0.31
26.45	hexyl acetate	methamyl acetate	142-92-7	0.33
28.37	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	0.16
32.93	nonanal	nonanaldehyde	124-19-6	0.18
38.68	2-methoxy-4-methylphenol	creosol	93-51-6	0.36
38.86	2-methoxy-5-methylphenol	5-methyl guaiacol	1195-09-1	0.29
84.22	hexadecanoic acid	palmitic acid	57-10-3	0.32
87.00	isopropyl palmitate	palmitic acid: isopropyl ester	142-91-6	trace
88.71	1-octadecanol	stearyl alcohol	112-92-5	0.75
105.48	squalene		111-02-4	31.92

Table 5.2-3a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

The "acceptable" Bakto Flavors Bourbon vanilla bean contains compounds typical of vanilla including 4-hydroxy-3-methoxybenzaldehyde (vanillin), acetic acid and furan-2-carbaldehyde (furfural). The "rejected" Bakto Flavors Bourbon vanilla bean contains elevated levels of 2-pentyl furan and several compounds not identified in the good sample including 3-methylbutan-1-ol (isoamyl alcohol), 2-methoxy-4-methylphenol (creosol) and 2-methoxy-5-methylphenol (5-methyl guaiacol). The presence of fusel alcohol such as 3-methylbutan-1-ol and 2-methylbutan-1-ol along with the lack of furan-2-carbaldehyde in the bad bean appear to support the theory that fermentation had occurred and further validates the findings of the SPME-GC-MS analysis. Curiously, vanillin was not detected in the bad Bakto Bourbon vanilla bean sample. This is most likely due to non-polar nature of the single phase adsorbent.

5.2.3 Dynamic headspace (purge-and-trap)

Purge and trap, or more commonly known as dynamic headspace, is a technique that uses a carrier gas either through pressure or vacuum to sweep the volatiles above a sample into a collective trap. In this experiment, which is described in section 4, the vacuum technique was used to pull the volatiles though a trap of Tenax TA. Air from the surrounding environment was pulled through a charcoal filter, across the sample and then

out through an inert support resulting in an enrichment of volatiles. The collection does involve intricate glassware and is bit more complicated that SPME or HSSE but is still relatively quick and easy to use.

The sample collected on the Tenax TA trap is different than SPME or HSSE in that is non-discriminatory. SPME and HSSE both showed biases towards polarity of the analytes. The Tenax TA resin does not have differential affinity for compounds of varying polarities. This is a true concentrated headspace without bias. However, it should be noted that solvents with three carbons or less will not be captured efficiently by Tenax TA.

Figures 5.2-5 and 5.2-6 show the total ion chromatograms for the "acceptable" nad "rejected" Bakto Flavors Bourbon vanilla beans. Table 5.2-5 is a list of compounds identified in the "acceptable" Bakto Flavors Bourbon vanilla bean. Table 5.2-6 lists the compounds identified in the "rejected" Bakto Flavors Bourbon vanilla bean. Both of these analyses were qualitative as there was no internal standard used and amounts listed are based solely on percent area of the integrated chromatogram.

A total of 24 compounds were identified in the "acceptable" Bakto Bourbon vanilla bean. The packaging contaminants identified in the SPME-GC-MS analysis were not found in the dynamic headspace-GC-MS analysis. 1-methyl-4-(1-methylethenyl)-cyclohexene (limonene) was present in both "acceptable" and "rejected" Bakto Flavors Bourbon vanilla bean samples. This is not considered to be a newly identified compound

in vanilla beans. Rather it is an artifact in the air during the exposure/concentration step in dynamic headspace. Additionally, 4-tert-butylcyclohexyl acetate was found in the "acceptable" Bakto Flavors vanilla bean and is considered to be a contaminant in this analysis. This compound is not found in nature and is a synthetic fragrance compound.

Ten new compounds were identified in the dynamic headspace-GC-MS analysis that have not been previously reported in vanilla beans including 4-methyl heptane, 2,4-dimethyl-4-heptanone, 2,4-dimethyl-1-heptene, 4-methyl octane, 2,6-dimethyl-1,7-octadiene, 3,7-dimethyl-1,6-octadiene, 4-octanone, 3-octanone, and (1,1-dimethylethyl) benzene. Table 5.2-5a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

The "acceptable" Bakto Flavors Bourbon vanilla bean contains compounds typical of vanilla including 4-hydroxy-3-methoxybenzaldehyde (vanillin), acetic acid and 2-methoxyphenol (guaiacol). The "rejected" Bakto Flavors Bourbon vanilla bean contained elevated levels of several fusel alcohols such as 3-methyl-2-butanol, 3-methyl-butan-1-ol, 2-methyl-butan-1-ol and 3-methyl-2-buten-1-ol. The presence of fusel alcohols along with the lack of furan-2-carbaldehyde in the bad bean appear to support the theory that fermentation had occurred and further validates the findings of the SPME-GC-MS and HSSE-GC-MS analyses. Oddly, the levels of vanillin were very similar in both samples with 0.82% for the "acceptable" bean and 0.70% for the "rejected" bean. This emphasizes the fact that each type of headspace experiment has its own nuances. If

only purge & trap analysis was performed to troubleshoot the off-odor in the “rejected” bean, the conclusion of similar vanillin levels would be erroneously drawn whereas the other two experiments indicated large differences.

Figure 5.2-5: Total Ion Chromatogram of "acceptable" Bakto Flavors Bourbon vanilla bean by Dynamic Headspace-GC-MS

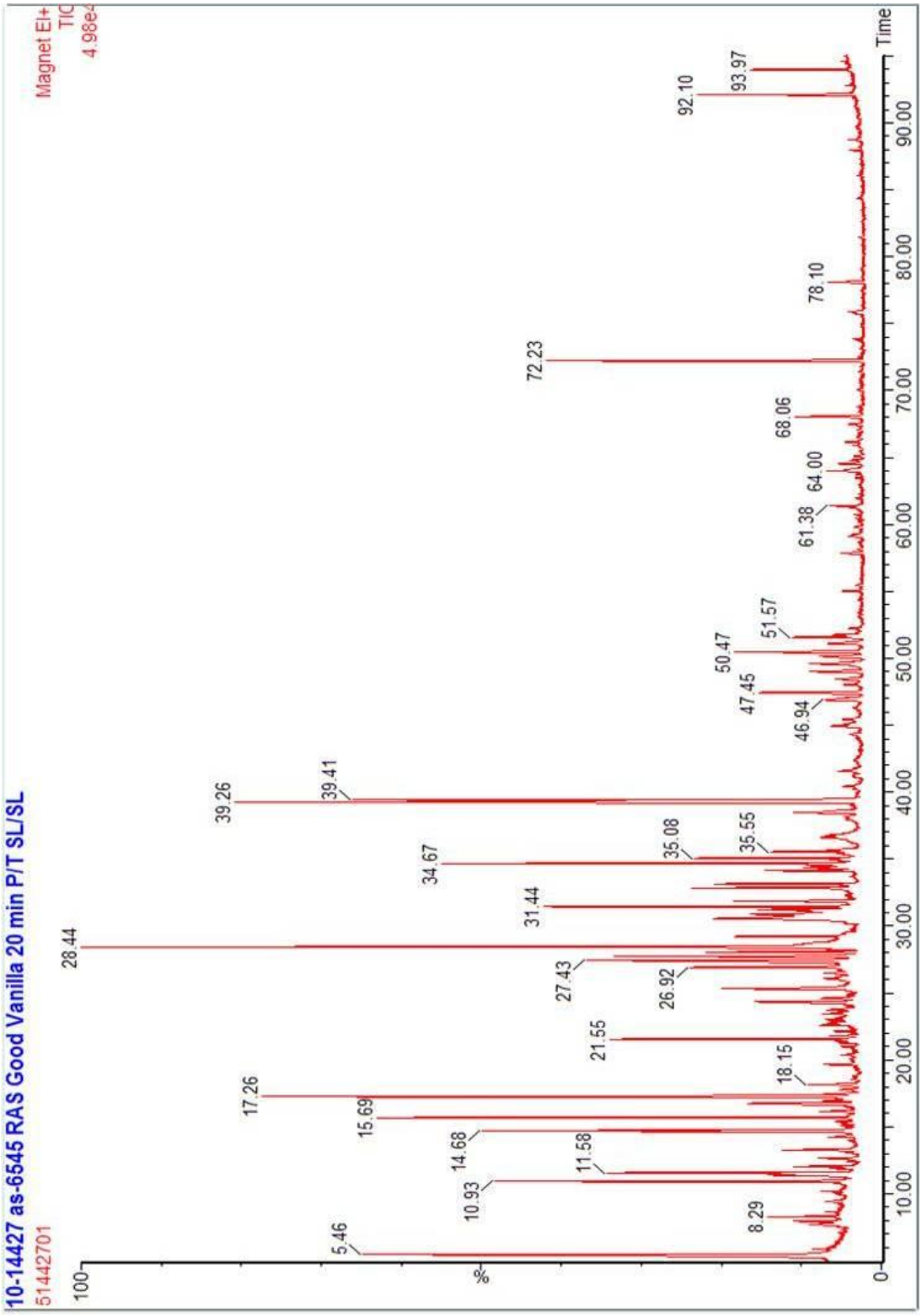

Table 5.2-5: Volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by Purge & Trap GC-MS.

Time	Assignment	Common Name	Registry #	% area
5.43	acetic acid	ethanoic acid	64-19-7	6.42
7.95	3-hydroxybutan-2-one	2-acetoin	513-86-0	0.57
10.92	methylbenzene	toluene	108-88-3	2.89
11.89	**4-methyl heptane**		**589-53-7**	**0.15**
13.28	octane		111-65-9	0.58
13.28	furan-2-carbaldehyde	furfural	98-01-1	trace
14.69	**2,6-dimethyl-4-heptanone**	**diisobutyl ketone**	**108-83-8**	**4.37**
15.70	**2,4-dimethyl-1-heptene**		**19549-87-2**	**3.52**
16.74	1,4-dimethylbenzene	p-xylene	106-42-3	1.25
17.27	**4-methyl octane**		**2216-34-4**	**6.13**
17.48	heptan-2-one	amyl methyl ketone	29308-56-3	0.25
21.55	benzaldehyde		100-52-7	2.19
21.55	**1,7-octadiene, 2,6-dimethyl**	**alpha-citronellene**	**6874-35-7**	**trace**
22.61	**3,7-dimethyl-1,6-octadiene**		**2436-90-0**	**0.27**
23.40	**4-octanone**	**butyl propyl ketone**	**589-63-9**	**trace**
23.75	phenol	phenyl alcohol, benzenol	108-95-2	0.24
24.34	**3-octanone**	**ethyl amyl ketone**	**106-68-3**	**1.20**
25.33	**(1,1-dimethylethyl)benzene**		**98-06-6**	**1.60**
27.73	1-methyl-4-propan-2-ylbenzene	cymene	99-87-6	2.26
28.44	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	8.29
29.23	1-phenylethanone	acetophenone	98-86-2	1.05
30.57	2-methoxyphenol	guaiacol	9009-62-5	2.95
46.91	4-tert-butylcyclohexyl acetate		32210-23-4	0.69
51.57	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	0.82

Table 5.2-5a: Newly identified volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by Purge & Trap GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
4-methyl heptane	cooked beef (Larick, 1990) dry cured ham (Berdague,1991)
2,6-dimethyl-4-heptanone	baked potatoes (Coleman, 1981)
2,4-dimethyl-1-heptene	Not found in VCF
4-methyl octane	Not found in VCF
1,7-octadiene, 2,6-dimethyl	Not found in VCF
3,7-dimethyl-1,6-octadiene	Not found in VCF
4-octanone	cooked beef (Herz, 1968) mushrooms (Vidal, 1986)
3-octanone	banana (Berger, 1986) guava (Idstein, 1985) mushrooms (Vidal, 1986)
(1,1-dimethylethyl)benzene	apple (Angelini, 1967) baked potates (Coleman, 1981)

Figure 5.2-6: Total Ion Chromatogram of "rejected" Bakto Flavors Bourbon vanilla bean by Dynamic Headspace-GC-MS

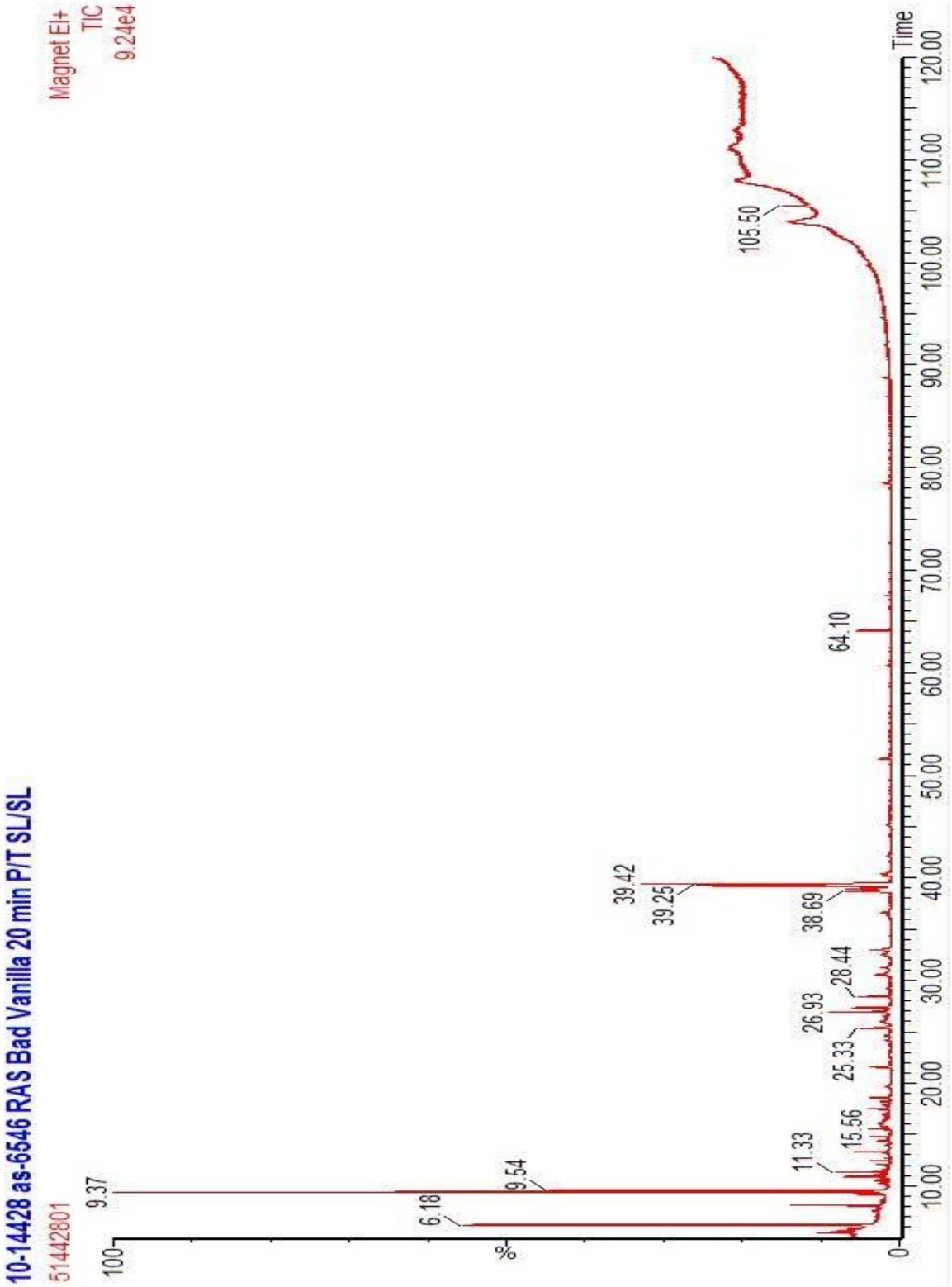

Table 5.2-6: Volatile and semi-volatile compounds identified in "rejected" Bakto Flavors Bourbon vanilla bean by Purge & Trap GC-MS.

Time	Assignment	Common Name	Registry #	% area
5.43	acetic acid	ethanoic acid	64-19-7	1.51
8.08	3-methyl-2-butanol		598-75-4	2.53
9.37	3-methyl-butan-1-ol	isopentanol	6423-06-9	21.99
9.54	2-methyl-butan-1-ol		137-32-6	9.78
10.26	2,4,5-trimethyl-1,3-dioxolane		3299-32-9	0.38
10.51	ethyl 2-methylpropanoate	ethyl isobutyrate	97-62-1	0.40
11.11	3-methyl-2-buten-1-ol		556-82-1	0.41
13.29	octane		111-65-9	0.94
14.34	1,3-octadiene		1002-33-1	0.35
15.56	ethyl-2-methylbuyrate		7452-79-1	0.53
16.11	ethyl 3-methylbutanoate	ethyl isovalerate	108-64-5	1.26
16.11	2,6-dimethyl-3,5-heptadien-2-ol		7411-76-8	trace
17.49	heptan-2-one	amyl methyl ketone	29308-56-3	0.69
21.56	benzaldehyde		100-52-7	0.85
25.33	2-pentylfuran	2-amyl furan	64079-01-2	1.34
28.44	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	1.31
30.56	2-methoxyphenol	guaiacol	9009-62-5	1.27
38.69	2-methoxy-5-methylphenol	5-methyl guaiacol	1195-09-1	2.68
38.95	2-methoxy-4-methylphenol	creosol	93-51-6	1.98
51.59	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	0.70

5.3 DTD/TDU GC-MS method

The sample introduction technique of Direct-Thermal-Desorption-Gas Chromatography-Mass Spectrometry is described in great detail by Hartman, et al. (Hartman, 1992). At the time this method was developed, the instrumentation used was on the cutting edge and offered the best in resolution and robustness. Several advancements in analytical instrumentation over the past two decades have offered opportunities to increase the resolution of this already sensitive headspace technique.

A block diagram of the initial instrumentation is shown in Figure 5.4-1. The sample of cryo-ground vanilla bean is inserted into a silanized glass lined stainless steel tube between glass wool plugs and thermally desorbed in the heater block at 220ºC in a stream of helium at constant pressure which also serves as the carrier gas for the gas chromatograph. The oven is cooled to -20ºC and is held at that temperature during the thermal desorption process. This serves as a cold zone to immobilize the eluted volatile and semi-volatile compounds from the sample. After desorption, the gas chromatograph oven containing a 50 meter polydimethylsiloxane (DB-1) column undergoes a temperature ramp to facilitate the elution of compounds in boiling point order. A heated transfer line from the gas chromatograph to the mass spectrometer is held at a constant temperature of 280ºC. The mass spectrometer scans masses from 33-350 once each second with a 0.8 second interscan time (Adedeji, 1993b). The scan rate was decreased to 0.6 although the interscan rate remained at 0.8 seconds and a 30 meter DB-5 column was used by Lee (2006).

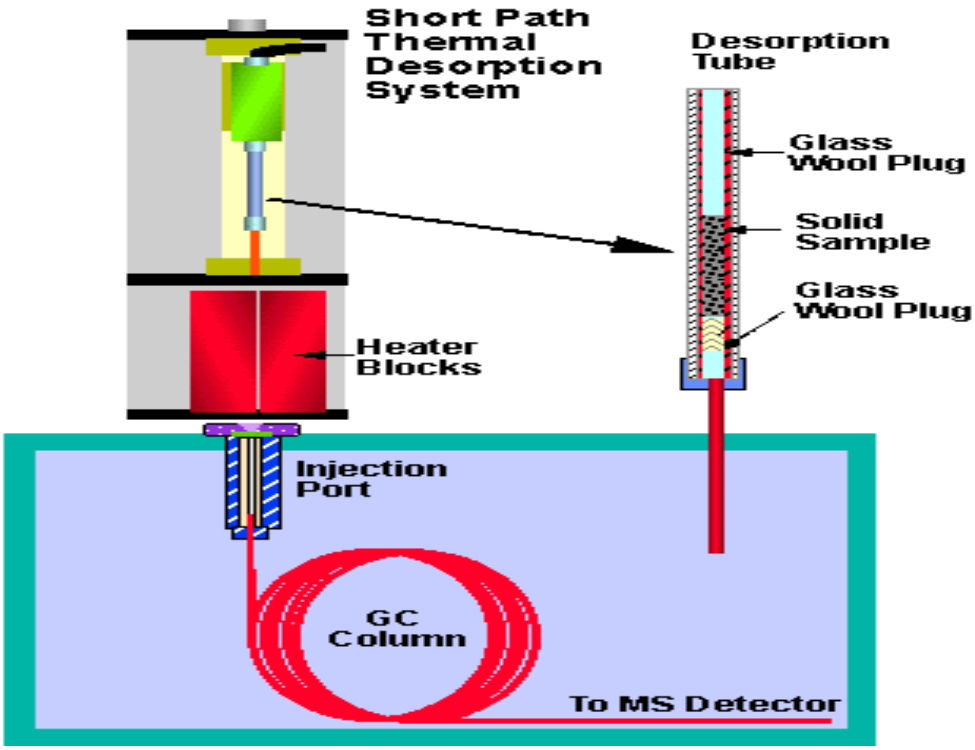

Figure 5.3-1: Original Short Path Thermal Desorption System

Source: Lee, 2006.

In the new DTD/TDU-GC-MS method, the sample introduction is very similar. An inert glass tube containing the cryo-ground sample between two plugs of glass wool is used. The glass tube is then thermally desorbed from an initial temperature of 30ºC to 275ºC at a rate of 60ºC/minute into a cryo-focusing GC inlet held at -120ºC using liquid nitrogen. Cryo focusing the inlet at such a low temperature allows the analytes to be trapped in a narrow band and also prevents break though. The thermal desorption occurs under a high linear velocity which improves the thermal release of analytes from the inert chromatographic support, but compounds are retained in the inlet. Introduction of the sample into the analytical column as a narrow band of analytes increases the resolution.

Older gas chromatograph systems, or constant pressure systems, relied on a preset carrier gas pressure in the inlet to move analytes through the analytical column. The disadvantage to these types of systems is that as the oven temperature increases to elute higher boiling compounds, the carrier gas becomes more viscous. The increased viscosity ultimately slows the linear velocity. Figure 5.3-2 shows the Van Deemter curve for common carrier gases used in gas chromatography. Helium demonstrates a minima on the curve at 20-35 cm/sec. The y-axis is a measurement of resolution in terms of the height of a theoretical plates (HETP) originating from distillation columns. The smaller

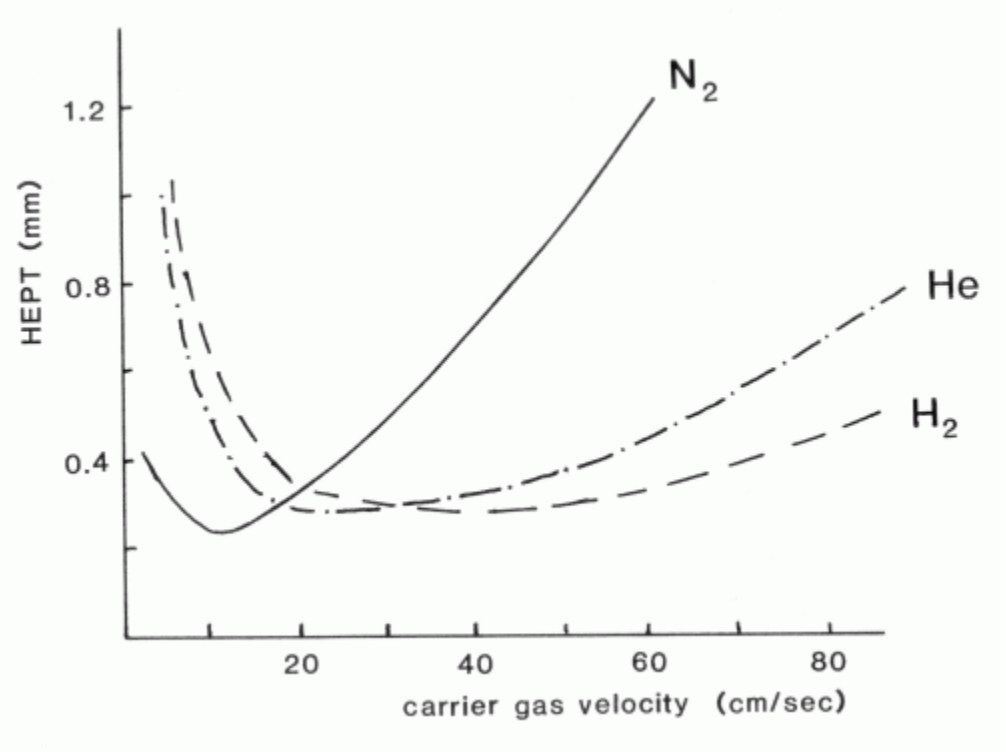

Figure 5.3-2: Plot of height of an effective theoretical plate (HETP) against carrier gas velocity (Van Deemter curve) for common carrier gases.

Source: www.agilent.com

the height of the theoretical plate, the larger the resolution. When a carrier gas loses linear velocity in the analytical column due to increases in viscosity, the effect on resolution can be extreme as the height of the theoretical plates rises exponentially as the line approaches the y-axis. The advent of electronic pneumatic (or pressure) control for gas chromatographs directly addresses this loss of resolution. The pressure in the inlet is set at a reference temperature to achieve optimal linear velocity. As the oven increases in temperature, the pressure in the inlet increases to maintain the linear velocity, and thus the resolution. This is often referred to as constant flow operation. The original experimentation by Hartman, Adedeji and Lee was done using a Varian 3400 gas chromatograph which pre-dated the existence of electronic pneumatic control of the carrier gas. The new DTD/TDU-GC-MS method uses an Agilent 6890 gas chromatograph which has the benefit of electronic pneumatic control.

The basic operation of a double focusing magnetic sector mass spectrometer has not changed very much over the years. However, advancements in electronic have allowed for fast scan rates and decreased interscan rates. This ultimately leads to faster data acquisition, more data points acquired per peak, and thus higher resolution. The original work done by Hartman, Adedeji and Lee was performed on a Finnigan Mat 8230 instrument. The new DTD/TDU-GC-MS method was performed on a Waters Micromass Auto Spec, which at the time of this development, is state of the art for mass spectrometers. The scan range is from mass 20 to 500 during the acquisition at a rate of 0.3sec with a 0.3 second interscan rate.

5.4 DTD/TDU-GC-MS results for vanilla beans

The direct thermal desorption method works well for introducing volatile and semi-volatile materials into the inlet. However, some of the higher boiling compounds are still elusive even though they have clean spectra due in part to the higher resolution of the new method. The mass spectral libraries do not contain a large amount of information on higher molecular weight compounds because they are not typically found in traditional GC-MS. A prime example of this is the region of 110 minutes and beyond for all of the DTD/TDU GC-MS runs. There are distinct peaks in the region, but the structures were not able to be determined by traditional library searches. These peaks are most likely a mixture of diketones and other high molecular weight compounds, some of which have been previously identified by DTD-GC-MS (Lee, 2006). The source of these diketones and other large species has been reported as the epicuticular wax of the vanilla beans (Ramaroson, 2000). These compounds are believed by perfumers to have aroma properties, but these compounds do not contribute to the aroma of vanilla extracts as there are too large to be extracted by ethanol. As more people begin to use whole vanilla beans and seeds for flavoring, it is possible that these compounds can add aroma and flavor impact in ways that vanilla extract cannot.

In each of the analyses, the compounds identified are listed in table format. The use of an internal standard in this desorption method allows the quantitation of identified compounds. Compounds highlighted in yellow are considered to be outside contaminants to the experiment. Compounds highlighted in green are newly identified

compounds in vanilla beans. Newly identified compounds were cross referenced with the Volatile Components in Foods (VCF) Database to identify those compounds that have been identified before in nature. Several compounds were not found in the VCF database. Although the omission of these compounds from the database indicates that they have not been previously reported, the validity of their identification in this analysis is not questioned. The VCF database is a comprehensive source of information, but is not exhaustive and new compounds are continuously added.

5.4.1 "Acceptable" Bakto Flavors Bean – *V. planifolia*

Figure 5.4-1 is the total ion chromatogram for the "acceptable" Bakto Flavors Bourbon vanilla bean. The list of identified compounds is shown in Table 5.4-1. A total of 74 compounds were identified in this analysis. The vanillin content in this bean was 1.20%. The analysis also revealed high amounts of acetic acid, 2-methoxyphenol, hydroxydihydromaltol, 5-(hydroxymethyl)furan-2-carbaldehyde, 4-hydroxybenzladehyde, vanillin, hexadecanoic acid and 1-octadecanol. The compounds identified for this Bourbon vanilla bean analysis are in line with previous data that has been reported in the literature (Klimes, 1976; Hartman, 1992; Adedeji, 1993b; Lee, 2006).

In Table 5.4-1, cells highlighted in green represent compounds reported in Bourbon vanilla beans for the first time including: acetone, 2-methyl propanal, 3-

hydroxy-3-penten-2-one, 2(5H)-furanone, 2-hydroxy-2-cyclopenten-1-one, 4-hydroxy-5-methyl-3(2H)-furanone, 2-furancarboxylic acid, lilial acid, 4-(4-hydroxyphenyl)-3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one (E), 2 isomers of vanillin glyceryl acetal, 1-octadecanol, ethyl heptadecanoate, ethyl octadecanoate, z-12-pentacosene and z-14 nonacosene. Table 5.4-1a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

Figure 5.4-1: Total Ion Chromatogram of "acceptable" Bakto Flavors Bourbon vanilla bean by TDU/DTD GC-MS

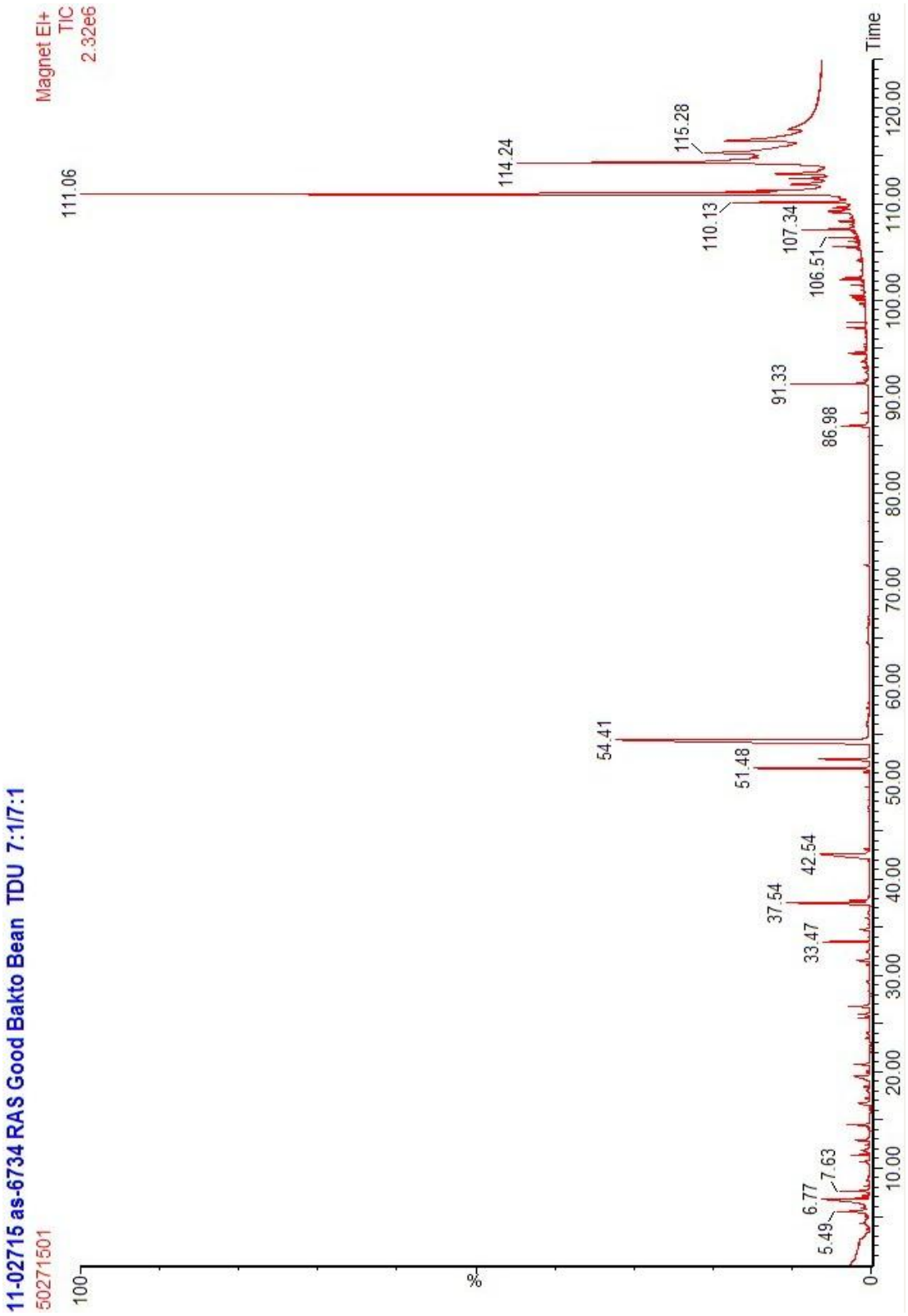

Table 5.4-1: Volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
3.66	acetaldehyde	ethanal	75-07-0	35
4.26	**acetone**		**67-64-1**	382
4.90	**2-methyl propanal**	**iso butyl aldehyde**	**78-84-2**	trace
5.07	butan-2-one	methyl ethyl ketone	78-93-3	trace
6.72	acetic acid	ethanoic acid	64-19-7	1671
6.95	3-methylbutanal	Isovaleraldehyde	590-86-3	89
7.12	3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	47
7.61	1-hydroxypropan-2-one	hydroxy acetone, pyruvic alcohol	116-09-6	481
8.17	pentane-2,3-dione	acetyl propionyl	600-14-6	40
10.80	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	30
11.37	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	272
12.40	2,3-butanediol	dimethylene glycol	513-85-9	65
13.35	**3-hydroxy 3-penten-2-one**		**52704-36-6**	trace
14.30	furan-3-ylmethanol	3-furanmethanol	4412-91-3	42
14.50	furan-2-carbaldehyde	furfural	98-01-1	363
16.73	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	448
17.25	cyclopent-4-ene-1,3-dione	4-cyclopentene-1,3-dione	930-60-9	69
18.46	**2(5H)-furanone**		**497-23-4**	96
20.77	**2-hydroxy-2-cyclopenten-1-one**		**10493-98-8**	271
23.49	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	66
25.97	phenol	phenyl alcohol, benzenol	108-95-2	171
29.34	**4-hydroxy-5-methyl-3(2H)-furanone**		**19322-27-1**	116
31.09	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	65
31.53	1-(2-furyl)-2-hydroxyethanone	2-(hydroxyacetyl)furan	17678-19-2	310
32.10	**2-furancarboxylic acid**	**2-furoic acid**	**88-14-2**	trace
32.80	3-methylphenol	cresol	108-39-4	trace
33.47	2-methoxyphenol	guaiacol	9009-62-5	697
35.20	nonanal	nonanaldehyde	124-19-6	trace
37.50	**4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl**	**hydroxydihydromaltol**	**28564-83-2**	2124
41.25	2-methoxy-4-methylphenol	creosol	93-51-6	trace
41.90	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	trace
42.52	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	2455
43.10	4-ethenylphenol	4-vinyl phenol	2628-17-3	86
47.00	(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	trace
49.54	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	62
50.00	deca-2,4-dienal	2,4-decadienal	2363-88-4	trace
51.05	4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	104

51.47	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
52.40	4-hydroxybenzaldehyde		123-08-0	1107
54.38	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	11982
57.70	4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol	498-00-0	45
58.40	methyl 4-hydroxybenzoate	methyl paraben	99-76-3	trace
59.70	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	trace
61.90	methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	trace
63.60	4-(4-hydroxyphenyl)-2-butanone	raspberry ketone	5471-51-2	trace
64.49	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	57
66.10	dodecanoic acid	lauric acid	143-07-7	trace
66.50	diethyl phthalate		84-66-2	trace
69.50	4-hydroxy-3,5-dimethoxybenzaldehyde	syringic aldehyde	134-96-3	trace
72.54	**4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid**	**lilial acid**	**66735-04-4**	91
72.75	4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	trace
73.25	**3-buten-2-one, 4-(4-hydroxyphenyl)**		**3160-35-8**	trace
77.15	tetradecanoic acid	myristic acid	544-63-8	trace
78.00	**4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)**		**22214-42-2**	trace
82.25	pentadecanoic acid	pentadecylic acid	1002-84-2	trace
82.75	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
83.25	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
85.65	methyl hexadecanoate	methyl palmitate	112-39-0	trace
85.85	dibutyl phthalate		84-74-2	trace
86.96	hexadecanoic acid	palmitic acid	57-10-3	567
88.31	ethyl hexadecanoate	ethyl palmitate	628-97-7	79
91.33	**1-octadecanol**	**stearyl alcohol**	**112-92-5**	915
91.68	**ethyl heptadecanoate**	**heptadecanoic acid ethyl ester**	**14010-23-2**	68
93.66	octadecanoic acid	stearic acid	57-11-4	63
94.60	**ethyl octadecanoate**	**ethyl stearate**	**111-61-5**	109
97.03	9-tricosene		27519-02-4	100
97.73	tricosane		638-67-5	168
100.0	tetracosane		646-31-1	69
101.6	**z-12-pentacosene**			123
102.2	pentacosane		629-99-2	169
102.4	diethylhexyl phthalate		117-81-7	124
104.2	hexacosane		630-01-3	36
106.1	heptacosane		593-49-7	98
108.2	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	135
109.3	**z-14 nonacosene**		**54863-80-8**	197

Table 5.4-1a: Newly identified volatile and semi-volatile compounds identified in "acceptable" Bakto Flavors Bourbon vanilla bean by TDU/DTD GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
acetone	apples (Mattheis, 1991) starfruit (MacLeod, 1990) Chinese quince (Mihara, 1987)
2-methyl propanal	black tea (Bondarovich, 1967) cocoa (Van Praag, 1968) mushrooms (Vidal, 1986) baked potato (Coleman, 1981)
3-hydroxy 3-penten-2-one	coffee (Kung, 1974)
2(5H)-furanone	roasted filberts (Kinlin, 1972) roasted peanuts (Ho, 1982)
2-hydroxy-2-cyclopenten-1-one	Not found in VCF
4-hydroxy-5-methyl-3(2H)-furanone	guava (Idstein, 1985) tomato (Buttery, 1995)
2-furancarboxylic acid	guava (Idstein, 1985) strawberry fruit (Mussinan, 1975)
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	rambutan (Ong, 1998)
4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid	Not found in VCF
3-buten-2-one, 4-(4-hydroxyphenyl)	Not found in VCF
4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
1-octadecanol	cherimoya (Idstein, 1984) cranberry (Croteau, 1968) guava (Idstein, 1985)
ethyl heptadecanoate	grilled beef (Hsu, 1982) cognac (Ledauphin, 2004)
ethyl octadecanoate	cognac (Ledauphin, 2004) strawberry guava (Pino, 2001)
z-12-pentacosene	Not found in VCF
z-14 nonacosene	Not found in VCF

5.4.2 "Rejected" Bakto Flavors Vanilla Bean – *V. planifolia*

Figure 5.4-2 is the total ion chromatogram for the "rejected" Bakto Flavors Bourbon vanilla bean. The list of identified compounds is shown in Table 5.4-2. A total of 74 compounds were identified in this analysis. The vanillin content in this bean was 0.10%. The analysis also revealed high amounts of 2-methoxyphenol, 2-methoxy-4-methylphenol, vanillin, hexadecanoic acid and 1-octadecanol.

Although several compounds were identified for the first time in this analysis, the vanilla bean was not in its native state and therefore these novel compounds cannot be considered as characteristic of top quality Bourbon vanilla beans.

Some of the major differences between the "acceptable" and "rejected" Bakto Flavors vanilla beans that the TDU/DTD GC-MS analysis exposed include the loss of vanillin in the "rejected" Bakto Flavors Bourbon sample as well as the increases of 2-methoxy-4-methylphenol and 2-methoxyphenol and the loss of hydroxydihydromaltol and hydroxy methyl furfural. The lack of a concentration step for TDU/DTD-GC-MS as compared to the other headspace techniques may explain the omission of the fusel alcohols that were present in the SPME, HSSE and Purge & Trap experiments. This reconfirms that each analytical technique has its own advantages and disadvantages. The loss of hydroxymethylfurfural and hydroxydihydromaltol in the "rejected" bean indicates that an organism may have been feeding on glucose, which is their primary food. The depletion of precursor molecules during the fermentation process could explain the loss

of hydroxymethylfurfural and hydroxydihydromaltol Additionally, the loss of furfural is another indicator of fermentation.

Figure 5.4-2: Total Ion Chromatogram of "rejected" Bakto Flavors Bourbon vanilla bean by TDU/DTD GC-MS

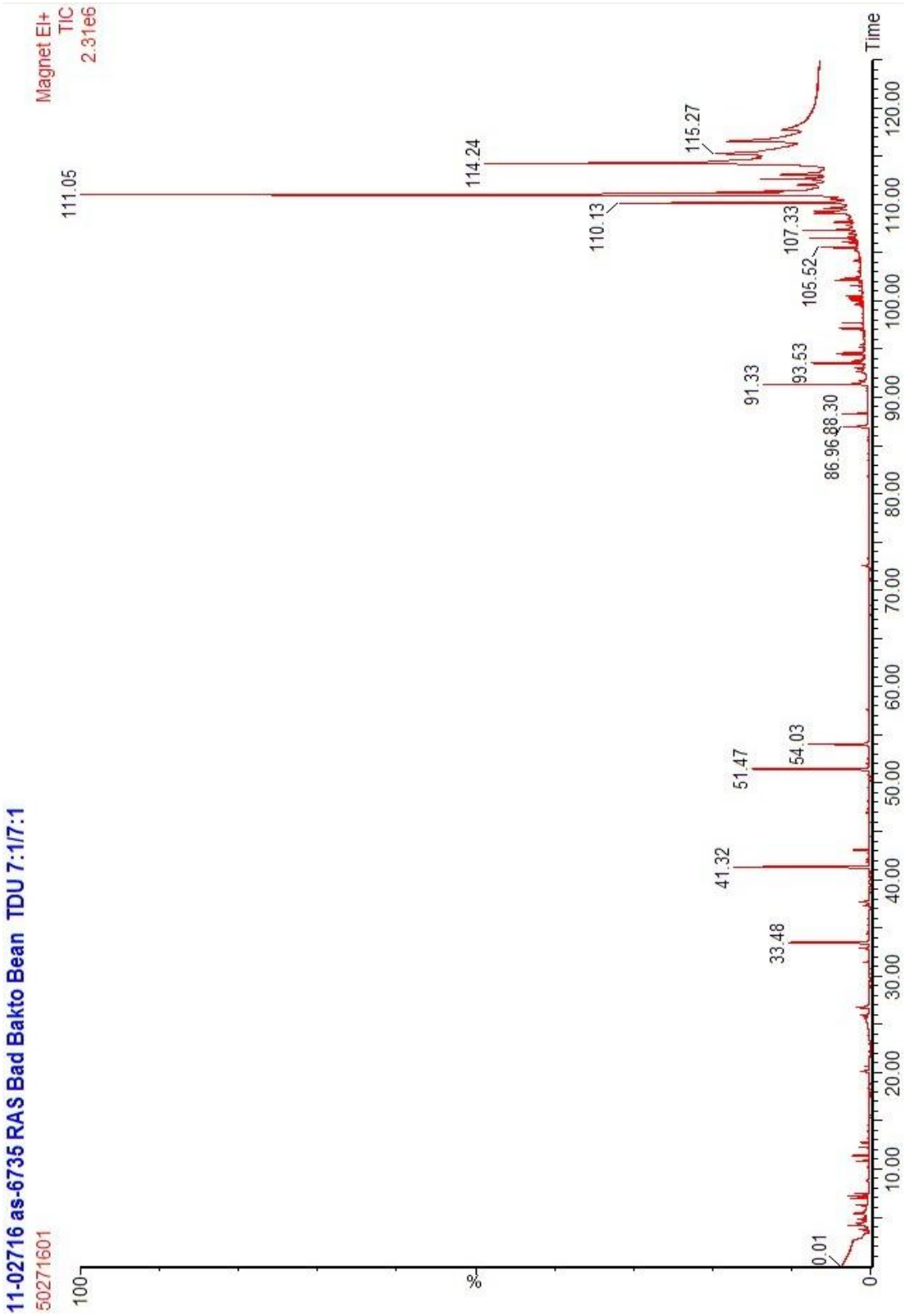

Table 5.4-2: Volatile and semi-volatile compounds identified in "rejected" Bakto Flavors Bourbon vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
6.26	acetic acid	ethanoic acid	64-19-7	177
6.98	3-methylbutanal	Isovaleraldehyde	590-86-3	107
7.16	3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	139
10.83	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	125
11.39	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	170
12.15	2,4-pentanedione		123-54-6	trace
12.24	2,3-butanediol	dimethylene glycol	513-85-9	87
13.25	hexanal		66-25-1	trace
14.00	1-hydroxy-2-pentanone		64502-89-2	trace
14.25	furan-3-ylmethanol	3-furanmethanol	4412-91-3	trace
14.50	furan-2-carbaldehyde	furfural	98-01-1	trace
15.60	1,3-octadiene		1002-33-1	trace
16.50	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	trace
17.25	cyclopent-4-ene-1,3-dione	4-cylopentene-1,3-dione	930-60-9	trace
18.39	2(5H)-furanone		497-23-4	34
19.10	2-heptanone		110-43-0	trace
20.64	2-hydroxy-2-cyclopenten-1-one		10493-98-8	69
25.93	phenol	phenyl alcohol, benzenol	108-95-2	205
29.25	4-hydroxy-5-methyl-3(2H)-furanone		19322-27-1	trace
30.90	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	trace
32.87	4-methyl phenol		106-44-5	175
33.47	2-methoxyphenol	guaiacol	9009-62-5	1471
34.38	2-nonanone		821-55-6	51
35.25	nonanal	nonanaldehyde	124-19-6	trace
35.50	2-nonanol		628-99-9	trace
37.30	2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one	hydroxydihydromaltol	8564-83-2	127
37.69	3,4-dimethylfuran-2,5-dione	dimethylmaleic anhydride	766-39-2	209
41.31	2-methoxy-4-methylphenol	creosol	93-51-6	2552
41.83	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	61
42.09	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	43
43.07	4-ethenylphenol	4-vinyl phenol	2628-17-3	257
46.30	1,4-benzendiol		123-31-9	trace
46.96	(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	55
48.10	4-methyl-1,2-benzenediol	4-methyl catechol	452-86-8	trace
49.54	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	48
50.80	4-hydroxy benzyl alcohol		623-05-2	trace

51.45	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
52.03	4-hydroxybenzlaldehyde		123-08-0	59
54.02	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	982
55.25	1,2,3-trimethoxy-5-methyl benzene		6443-69-2	trace
57.30	4-butyl phenol		1638-22-8	trace
57.61	vanillyl alcohol		498-00-0	47
58.25	methyl 4-hydroxybenzoate	methyl paraben	99-76-3	trace
59.65	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	trace
62.30	vanillyl methyl ketone		2503-46-0	trace
63.60	4-(4-hydroxyphenyl)-2-butanone	raspberry ketone	5471-51-2	trace
66.00	dodecanoic acid	lauric acid	143-07-7	trace
66.50	diethyl phthalate		84-66-2	trace
69.25	4-(4-hydroxy-3-methoxyphenyl)-2-butanaone	zingerone	122-48-5	trace
72.55	4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid	lilial acid	66735-04-4	148
77.10	tetradecanoic acid	myristic acid	544-63-8	trace
79.10	ethyl octadecanoate		111-61-5	trace
81.78	6,10,14-trimethyl-2-pentadecanone	hexahydrofarnesyl acetone	502-69-2	31
82.20	1-hexadecene,7,11,15-trimethyl-3-methylene	neophytadiene	504-96-1	trace
85.50	methyl hexadecanoate	methyl palmitate	112-39-0	trace
85.75	dibutyl phthalate		84-74-2	trace
86.95	hexadecanoic acid	palmitic acid	57-10-3	532
88.30	ethyl hexadecanoate	ethyl palmitate	628-97-7	274
89.50	propan-2-yl hexadecanoate	isopropyl palmitate	142-91-6	trace
91.33	1-octadecanol		112-92-5	1371
91.67	9,12-octadecadienoic acid, methyl ester		112-63-0	114
91.78	heptadecanoic acid, ethyl ester		14010-23-2	26
93.54	ethyl (9Z,12Z)-octadeca-9,12-dienoate	ethyl linoleate	544-35-4	576
93.78	ethyl octadec-9-enoate	ethyl oleate	111-62-6	123
94.59	octadecanoic acid ethyl ester		111-61-5	184
97.03	9-tricosene		27519-02-4	213
97.73	tricosane		638-67-5	178
100.03	tetracosane		646-31-1	102
100.47	dipropylene glycol dibenzoate		20109-39-1	229
102.16	pentacosane		629-99-2	198
102.35	diethylhexyl phthalate		117-81-7	119
104.17	hexacosane		630-01-3	50
106.06	heptacosane		593-49-7	140
108.17	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	157
109.32	z-14 nonacosene		54863-80-8	336

5.4.3 Bourbon Bean – International Flavors & Fragrances – *V. planifolia*

Figure 5.4-3 is the total ion chromatogram for the Bourbon vanilla bean from International Flavors & Fragrances. The list of identified compounds is shown in Table 5.4-3. A total of 67 compounds were identified in this analysis. The vanillin content in this bean was 2.09%. The analysis also revealed high amounts of acetic acid, 3-methylbutanal, 3-methylbutan-2-one, 3-methylpentanal, furfural, furfuryl alcohol, 2-methoxyphenol, hydroxydihydromaltol, 5-(hydroxymethyl)-furan-2-carbaldehyde, 4-hydroxybenzladehyde and vanillin. The compounds identified for this Bourbon vanilla bean analysis are in line with previous data that has been reported in the literature (Klimes, 1976; Hartman, 1992; Adedeji, 1993b; Lee, 2006).

In Table 5.4-1, cells highlighted in green represent compounds reported in Bourbon vanilla beans for the first time including: acetone, 2-methyl propanal, 4-methyl-2-pentanone, 1-hydroxy-3-methyl-2-butanone, 1,3-octadiene, 2-hydroxy-2-cyclopenten-1-one, 2-acetyl-2-hydroxy-gamma-butyrolactone, lilial acid, 4-(4-hydroxyphenyl)-3-buten-2-one, coniferyl aldehyde, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one (E), 2 isomers of vanillin glyceryl acetal, 1-octadecanol and z-12-pentacosene. Table 5.4-3a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database this analysis is not questioned.

Figure 5.4-3: Total Ion Chromatogram of IFF Bourbon vanilla bean by TDU/DTD GC-MS

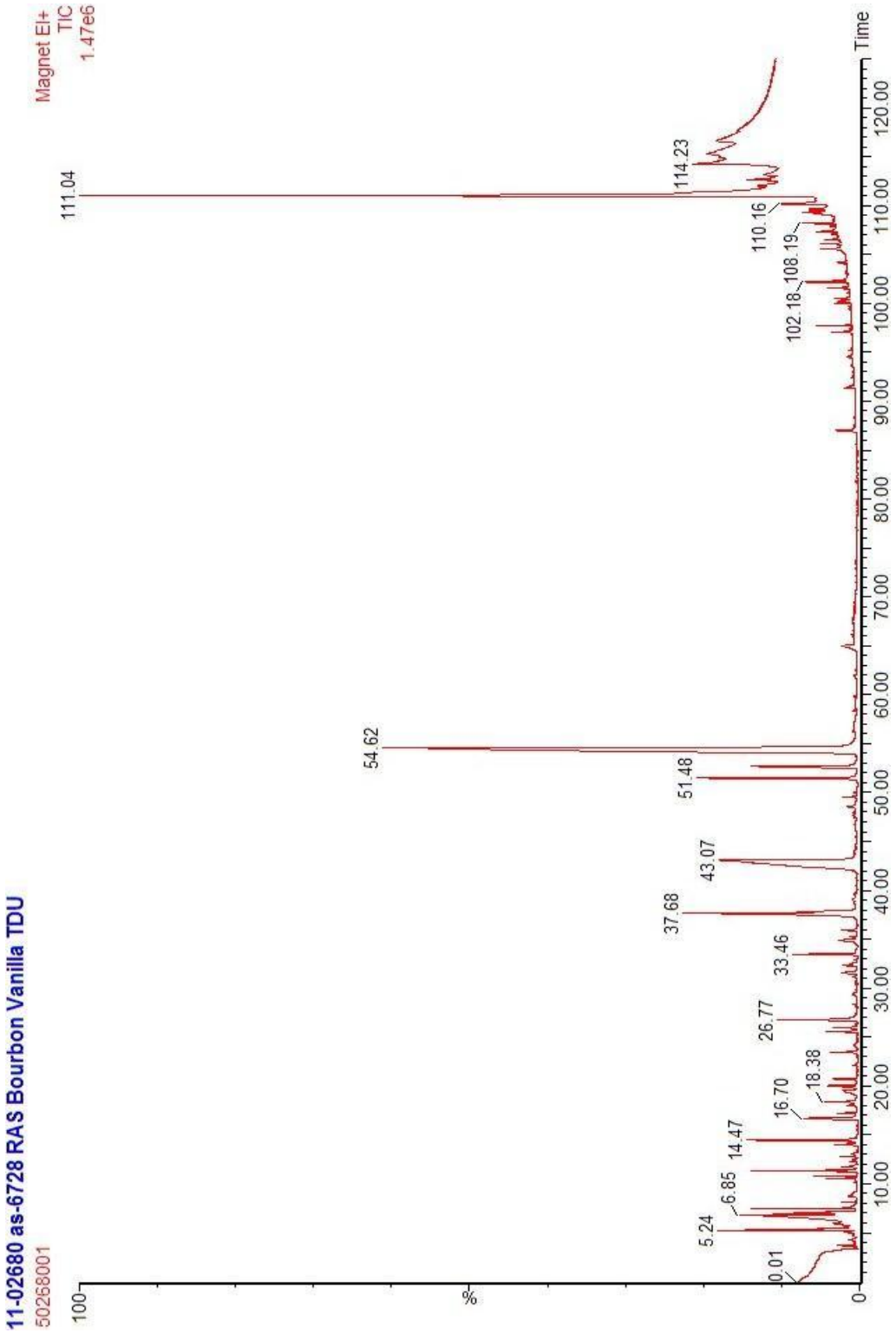

Table 5.4-3: Volatile and semi-volatile compounds identified in IFF Bourbon vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
3.69	acetaldehyde		75-07-0	159
4.26	**acetone**		**67-64-1**	96
4.96	**2-methyl propanal**	**iso butyl aldehyde**	**78-84-2**	33
5.38	butane-2,3-dione	diacetyl	431-03-8	175
6.79	acetic acid	ethanoic acid	64-19-7	4071
6.90	3-methylbutanal	Isovaleraldehyde	590-86-3	492
7.09	3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	533
8.14	pentane-2,3-dione	acetyl propionyl	600-14-6	86
10.10	4-methyl-2-pentanone	isobutyl methyl ketone	108-10-1	trace
10.76	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	265
11.34	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	1030
13.08	hexanal	hexanaldehdye	66-25-1	96
13.99	**1-hydroxy-3methyl-2-butanone**		**36960-22-2**	254
14.47	furan-2-carbaldehyde	furfural	98-01-1	1288
15.60	**1,3-octadiene**		**1002-33-1**	trace
16.68	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	882
17.21	cyclopent-4-ene-1,3-dione	4-cyclopentene-1,3-dione	930-60-9	205
20.72	**2-hydroxy-2-cyclopenten-1-one**		**10493-98-8**	247
23.46	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	334
25.96	phenol	phenyl alcohol, benzenol	108-95-2	249
31.16	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	59
32.33	methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	611-13-2	170
32.90	3-methylphenol	cresol	108-39-4	33
33.46	2-methoxyphenol	guaiacol	9009-62-5	729
34.90	**2-acetyl-2-hydroxy-gamma-butyrolactone**		**135366-64-2**	331
35.24	nonanal		124-19-6	50
37.65	2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one	hydroxydihydromaltol	8564-83-2	4452
41.90	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	trace
43.03	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	8409
49.55	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	128
50.01	deca-2,4-dienal	2,4-decadienal	2363-88-4	26
51.47	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
52.67	4-hydroxybenzaldehyde		123-08-0	1900
54.58	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	20940
58.38	methyl 4-hydroxybenzoate	methyl paraben	99-76-3	32
59.84	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	25

61.99	4-hydroxy benzoic acid	p-hydroxybenzoic acid	99-96-7	trace
62.50	1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2503-46-0	trace
64.99	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	437
66.50	diethyl phthalate		84-66-2	trace
69.50	4-hydroxy-3,5-dimethoxy-benzaldehyde		134-96-3	trace
72.50	**4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid**	**lilial acid**	**66735-04-4**	trace
72.75	4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	trace
73.25	**3-buten-2-one, 4-(4-hydroxyphenyl)**		**3160-35-8**	trace
73.50	**4-hydoxy-3-methoxycinnamaldehyde**	**coniferyl aldehyde**	**458-36-6**	trace
77.25	tetradecanoic acid	myristic acid	544-63-8	trace
78.00	**4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)**		**22214-42-2**	trace
81.80	6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	502-69-2	19
82.80	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
83.25	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
85.50	methyl palmitate		112-39-0	trace
85.80	dibutyl phthalate			trace
87.00	hexadecanoic acid	palmitic acid	57-10-3	308
88.25	ethyl hexadecanoate	ethyl palmitate	628-97-7	trace
91.35	**1-octadecanol**	**stearyl alcohol**	**112-92-5**	87
91.50	methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0	60
93.67	octadecanoic acid	stearic acid	57-11-4	22
95.21	docosane		629-97-0	25
97.04	9-tricosene		27519-02-4	137
97.74	tricosane		638-67-5	224
100.0	tetracosane		646-31-1	98
100.2	dipropylene glycol dibenzoate		20109-39-1	150
101.6	**z-12-pentacosene**			144
102.2	pentacosane		629-99-2	283
102.4	diethylhexyl phthalate		117-81-7	147
104.2	hexacosane		630-01-3	62
106.1	heptacosane		593-49-7	182
108.2	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	230

Table 5.4-3a: Newly identified volatile and semi-volatile compounds identified in IFF Bourbon vanilla bean by TDU/DTD GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
acetone	apples (Mattheis, 1991) starfruit (MacLeod, 1990) chinese quince (Mihara, 1987)
2-methyl propanal	black tea (Bondarovich, 1967) cocoa (Van Praag, 1968) mushrooms (Vidal, 1986) baked potato (Coleman, 1981)
1-hydroxy-3methyl-2-butanone	Not found in VCF
1,3-octadiene	grilled beef (Hsu, 1982) heated soybean (Del Rosario, 1984)
2-hydroxy-2-cyclopenten-1-one	Not found in VCF
2-acetyl-2-hydroxy-gamma butyrolactone	Not found in VCF
4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid	Not found in VCF
3-buten-2-one, 4-(4-hydroxyphenyl)	Not found in VCF
4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
1-octadecanol	cherimoya (Idstein, 1984) cranberry (Croteau, 1968) guava (Idstein, 1985)
z-12-pentacosene	Not found in VCF

5.4.4 Indonesian Bean – International Flavors & Fragrances – *V. planifolia*

Figure 5.4-4 is the total ion chromatogram for the Indonesian vanilla bean from International Flavors & Fragrances. The list of identified compounds is shown in Table 5.4-4. A total of 72 compounds were identified in this analysis. The vanillin content in this bean was low at 0.75%, which is typical for Indonesian vanilla beans (Lee, 2006). The analysis did reveal high amounts of acetic acid, 3-methylpentanal, hexanal, furfuryl alcohol, 2-methoxyphenol, hydroxydihydromaltol, 3,4-dimethylfuran-2,5-dione, hydroxyl methyl furfural, vanillin and hexadecanoic acid. The compounds identified for this Indonesian vanilla bean analysis are in line with previous data that has been reported in the literature (Lee, 2006).

In Table 5.4-4, cells highlighted in green represent compounds reported in Indonesian vanilla beans for the first time including: 4-methyl-2-pentanone, 2-hydroxy-2-cyclopenten-1-one, 4-methyl-2-pentenoic acid, 2-acetyl-2-hydroxy-gamma-butyrolactone, 4-(4-hydroxyphenyl)-3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one (E), 2 isomers of vanillin glyceryl acetal and z-12-pentacosene. Table 5.4-4a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

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Figure 5.4-4: Total Ion Chromatogram of IFF Indonesian vanilla bean by TDU/DTD GC-MS

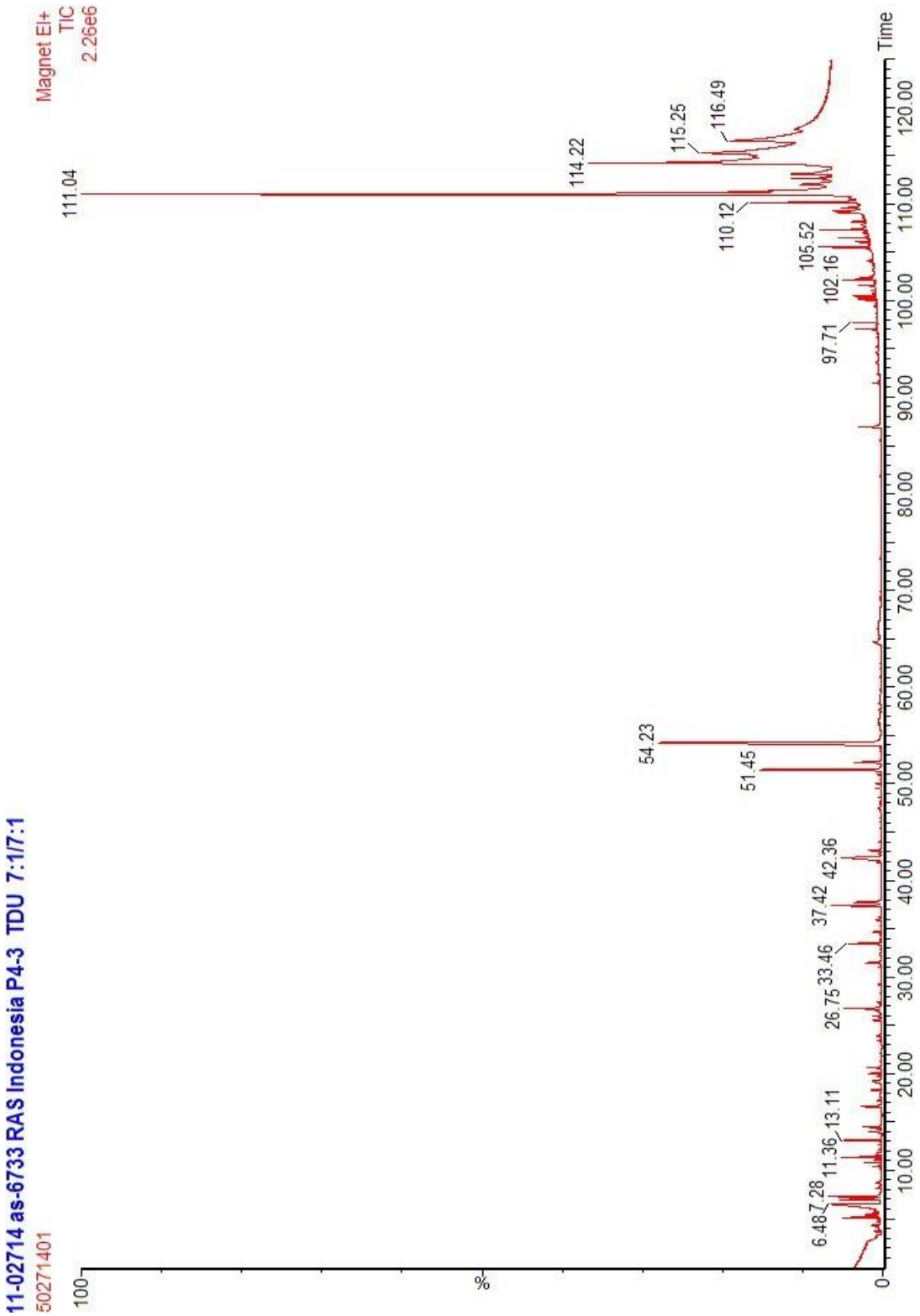


Table 5.4-4: Volatile and semi-volatile compounds identified in IFF Indonesian vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
3.67	acetaldehyde	ethanal	75-07-0	76
5.39	butane-2,3-dione	diacetyl	431-03-8	113
6.43	acetic acid	ethanoic acid	64-19-7	1844
6.94	3-methylbutanal	Isovaleraldehyde	590-86-3	308
7.11	3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	204
8.17	pentane-2,3-dione	acetyl propionyl	600-14-6	71
8.20	pentanal	valeraldehyde	110-62-3	trace
10.20	**4-methyl-2-pentanone**	**isobutyl methyl ketone**	**108-10-1**	trace
10.80	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	162
11.37	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	484
12.16	2,3-butanediol	dimethylene glycol	513-85-9	37
13.12	hexanal	hexanaldehdye	66-25-1	431
14.49	furan-2-carbaldehyde	furfural	98-01-1	260
16.61	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	407
17.24	cyclopent-4-ene-1,3-dione	4-cyclopentene-1,3-dione	930-60-9	69
20.63	**2-hydroxy-2-cyclopenten-1-one**		**10493-98-8**	228
23.25	**4-methyl-2-pentenoic acid**		**10321-71-8**	trace
23.47	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	66
25.95	phenol	phenyl alcohol, benzenol	108-95-2	112
26.50	hexanoic acid	caproic acid	142-62-1	trace
27.37	2-pentyl furan		3777-69-3	51
31.03	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	41
32.80	3-methylphenol	cresol	108-39-4	trace
33.46	2-methoxyphenol	guaiacol	9009-62-5	556
34.67	**2-acetyl-2-hydroxy-gamma-butyrolactone**		**135366-64-2**	155
35.25	nonanal		124-19-6	trace
37.41	2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one	hydroxydihydromaltol	8564-83-2	963
37.73	3,4-dimethylfuran-2,5-dione	dimethylmaleic anhydride	766-39-2	486
41.25	2-methoxy-4-methylphenol	creosol	93-51-6	trace
41.88	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	98
42.35	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	1447
43.08	4-ethenylphenol	4-vinyl phenol	2628-17-3	157
46.90	(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	trace
49.52	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	77
50.00	deca-2,4-dienal	2,4-decadienal	2363-88-4	77
50.96	4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	73
51.44	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
52.22	4-hydroxybenzaldehyde		123-08-0	480

54.23	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	7484
57.70	4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol	498-00-0	trace
58.20	methyl 4-hydroxybenzoate	methyl paraben	99-76-3	trace
59.70	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	trace
61.90	methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	trace
62.25	1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2503-46-0	trace
64.69	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	226
66.10	dodecanoic acid	lauric acid	143-07-7	trace
66.50	diethyl phthalate		84-66-2	trace
72.75	4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	trace
73.25	**3-buten-2-one, 4-(4-hydroxyphenyl)**		**3160-35-8**	trace
77.15	tetradecanoic acid	myristic acid	544-63-8	trace
78.00	**4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)**		**22214-42-2**	trace
81.78	6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	502-69-2	trace
82.25	pentadecanoic acid	pentadecylic acid	1002-84-2	trace
82.75	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
83.25	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
85.65	methyl hexadecanoate	methyl palmitate	112-39-0	trace
85.85	dibutyl phthalate			trace
86.93	hexadecanoic acid	palmitic acid	57-10-3	431
88.31	ethyl hexadecanoate	ethyl palmitate	628-97-7	trace
91.47	methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0	58
93.61	octadecanoic acid	stearic acid	57-11-4	47
95.25	docosane		629-97-0	trace
97.02	9-tricosene		27519-02-4	171
97.71	tricosane		638-67-5	212
100.0	tetracosane		646-31-1	83
100.5	dipropylene glycol dibenzoate		20109-39-1	316
101.6	**z-12-pentacosene**			155
102.2	pentacosane		629-99-2	281
102.3	diethylhexyl phthalate		117-81-7	174
104.2	hexacosane		630-01-3	53
106.1	heptacosane		593-49-7	133
108.2	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	122
109.6	nonacosane		630-03-5	213

Table 5.4-4a: Newly identified volatile and semi-volatile compounds identified in IFF Indonesian vanilla bean by TDU/DTD GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
4-methyl-2-pentanone	black tea (Mick, 1984) Chinese quince (Mihara, 1987) cognac (Ledauphin, 2004) bourbon vanilla (Klimes, 1976)
2-hydroxy-2-cyclopenten-1-one	Not found in VCF
4-methyl-2-pentenoic acid	black tea (Mick, 1984)
2-acetyl-2-hydroxy-gamma butyrolactone	Not found in VCF
3-buten-2-one, 4-(4-hydroxyphenyl)	Not found in VCF
4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
z-12-pentacosene	Not found in VCF

5.4.5 Ugandan Bean – International Flavors & Fragrances – *V. planifolia*

Figure 5.4-5 is the total ion chromatogram for the Ugandan vanilla bean from International Flavors & Fragrances. The list of identified compounds is shown in Table 5.4-5. A total of 66 compounds were identified in this analysis. The vanillin content in this bean was 2.07%. The analysis also revealed high amounts of acetic acid, 3-methylbutanal, 3-methylpentanal, furfural, furfuryl alcohol, 2-methoxyphenol, hydroxydihydromaltol, hydroxyl methyl furfural, 4-hydroxybenzldehyde, vanillin and vanillic acid. There has only been one previously published study on the volatile and semi-volatile compounds in Ugandan vanilla beans and the current findings concur with the literature (Lee, 2006).

In Table 5.4-5, cells highlighted in green represent compounds reported in Ugandan vanilla beans for the first time including: 2(5H)-furanone, 2-hydroxy-2-cyclopenten-1-one, 2-acetyl-2-hydroxy-gamma-butyrolactone, 3,5-dihydroxy-2-methylpyran-4-one, 3-phenyl-2-propenoic acid, 4-hydroxy-2-methoxycinnamaldehyde, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one (E), 2 isomers of vanillin glyceryl acetal, kaurene, and z-12-pentacosene. Table 5.4-5a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

Figure 5.4-5: Total Ion Chromatogram of IFF Ugandan vanilla bean by TDU/DTD GC-MS

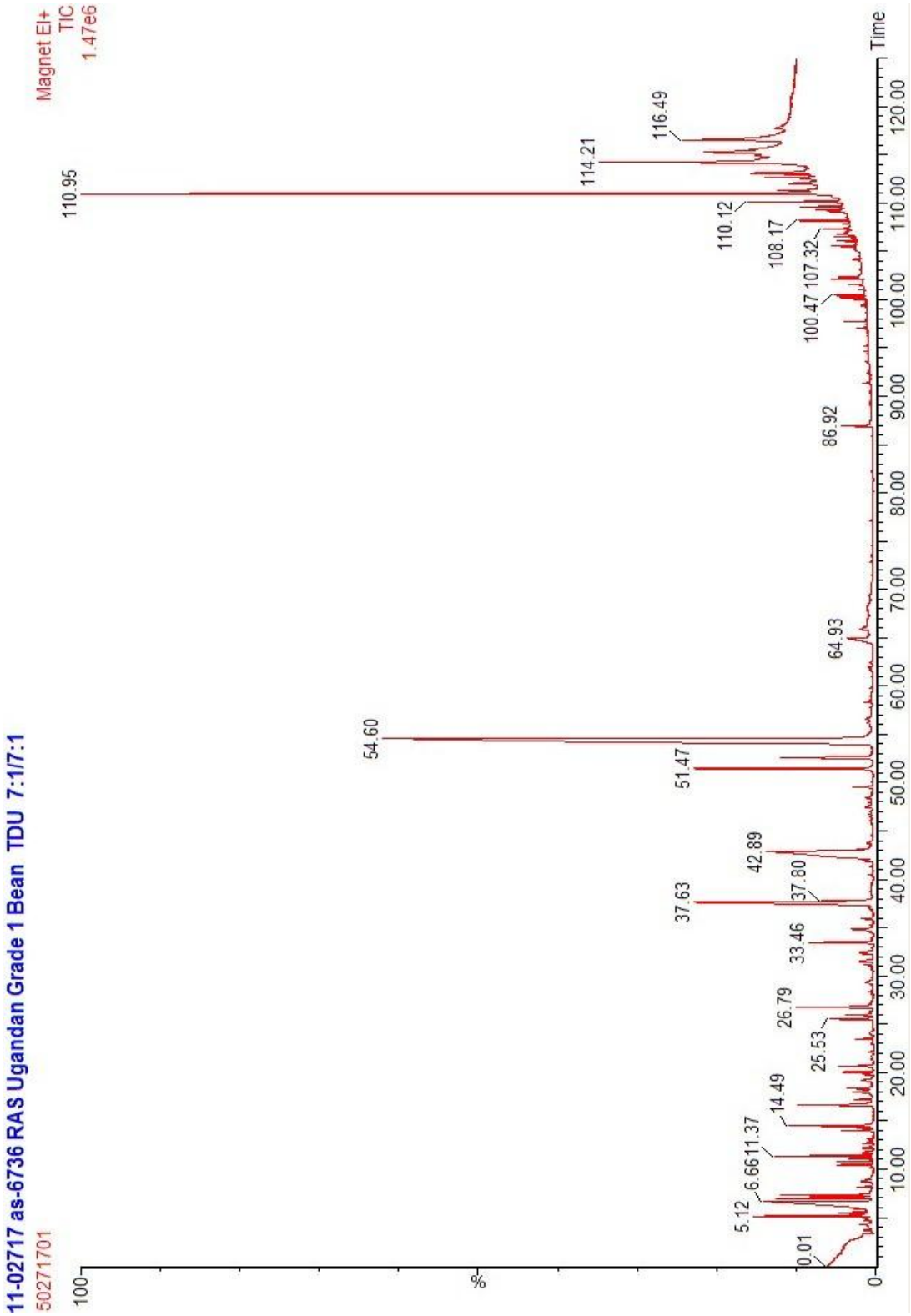


Table 5.4-5: Volatile and semi-volatile compounds identified in IFF Ugandan vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
3.67	acetaldehyde	ethanal	75-07-0	74
6.61	acetic acid	ethanoic acid	64-19-7	3806
6.97	3-methylbutanal	Isovaleraldehyde	590-86-3	579
7.12	3-methylbutan-2-one	isopropyl methyl ketone	563-80-4	293
8.17	pentane-2,3-dione	acetyl propionyl	600-14-6	76
10.81	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	208
11.37	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	687
12.20	2,3-butanediol	dimethylene glycol	513-85-9	88
13.12	hexanal	hexanaldehdye	66-25-1	74
13.99	1-hydroxy-2-pentanone		64502-89-2	307
14.49	furan-2-carbaldehyde	furfural	98-01-1	762
16.64	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	954
17.24	cyclopent-4-ene-1,3-dione	4-cyclopentene-1,3-dione	930-60-9	165
18.35	**2(5H)-furanone**		**497-23-4**	213
20.67	**2-hydroxy-2-cyclopenten-1-one**		**10493-98-8**	351
23.47	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	155
25.96	phenol	phenyl alcohol, benzenol	108-95-2	273
31.15	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	125
32.33	methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	611-13-2	135
32.80	3-methylphenol	cresol	108-39-4	trace
33.46	2-methoxyphenol	guaiacol	9009-62-5	693
34.52	3-hydroxy-2-methylpyran-4-one	maltol	118-71-8	49
34.84	**2-acetyl-2-hydroxy-gamma-butyrolactone**		**135366-64-2**	432
37.59	2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one	hydroxydihydromaltol	8564-83-2	3832
40.45	**3,5-dihydroxy-2-methylpyran-4-one**	**5-hydroxymaltol**	**1073-96-7**	69
41.92	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	144
42.85	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	5376
43.11	4-ethenylphenol	4-vinyl phenol	2628-17-3	277
46.98	(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	31
49.54	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	190
51.24	4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	20
51.45	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
52.60	4-hydroxybenzaldehyde		123-08-0	1424
54.55	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	20692
56.63	**3-phenyl-2-propenoic acid**	**cinnamic acid**	**621-82-9**	65
58.34	methyl 4-hydroxybenzoate	methyl paraben	99-76-3	99

59.80	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	19
61.96	methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	67
62.46	1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2503-46-0	36
64.90	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	834
66.10	dodecanoic acid	lauric acid	143-07-7	trace
69.39	4-hydroxy-3,5-dimethoxybenzaldehyde	syringic aldehyde	134-96-3	46
72.75	4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	63253-24-7	trace
73.60	**4-hydroxy-2-methoxycinnamaldehyde**		**127321-19-1**	trace
77.12	tetradecanoic acid	myristic acid	544-63-8	24
78.00	**4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)**		**22214-42-2**	trace
82.25	pentadecanoic acid	pentadecylic acid	1002-84-2	trace
82.75	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
83.25	**2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer**	**(vanillin glyceryl acetal)**		trace
85.65	methyl hexadecanoate	methyl palmitate	112-39-0	trace
85.85	dibutyl phthalate		84-74-2	trace
86.91	hexadecanoic acid	palmitic acid	57-10-3	386
90.25	**kaurene**		**562-28-7**	trace
91.48	methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0	23
93.59	octadecanoic acid	stearic acid	57-11-4	16
97.02	9-tricosene		27519-02-4	71
97.73	tricosane		638-67-5	127
99.39	hexanedioic acid, dioctyl ester	dioctyl adipate	123-79-5	30
100.2	tetracosane		646-31-1	268
100.5	dipropylene glycol dibenzoate		20109-39-1	273
101.6	**z-12-pentacosene**			103
102.2	pentacosane		629-99-2	187
102.3	diethylhexyl phthalate		117-81-7	190
104.2	hexacosane		630-01-3	43
106.1	heptacosane		593-49-7	108
108.2	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	285
109.6	nonacosane		630-03-5	310

Table 5.4-5a: Newly identified volatile and semi-volatile compounds identified in IFF Ugandan vanilla bean by TDU/DTD GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
2(5H)-furanone	roasted filberts (Kinlin, 1972) roasted peanuts (Ho, 1982)
2-hydroxy-2-cyclopenten-1-one	Not found in VCF
2-acetyl-2-hydroxy-gamma-butyrolactone	Not found in VCF
3,5-dihydroxy-2-methylpyran-4-one	honey
3-phenyl-2-propenoic acid	guava (Idstein, 1985) rambutan, (Ong, 1998) strawberry fruit (Mussinan, 1975)
4-methoxybenzeneacetic acid	Not found in VCF
4-hydroxy-2-methoxycinnamaldehyde	Not found in VCF
4-(4-hydroxy-3-methoxyphenyl) 3-buten-2-one (E)	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxan-5-ol isomer	Not found in VCF
kaurene	cranberry (Anjou, 1967)
z-12-pentacosene	Not found in VCF

5.4.6 Tahitian Bean – International Flavors & Fragrances – *V. tahitensis*

Figure 5.4-6 is the total ion chromatograms for the Tahitian vanilla bean from International Flavors & Fragrances. The list of identified compounds is shown in Table 5.4-6. A total of 65 compounds were identified in this analysis. The vanillin content in this bean was 0.93% which in agreement with previously published studies (Adedeji, 1993b; Lee, 2006). The analysis also revealed high amounts of acetic acid, 3-methylpentan-2-one, 3-methylpentanal, furfural, furfuryl alcohol, phenol, hydroxydihydromaltol, hydroxyl methyl furfural, anisyl alcohol, 4-hydroxybenzaldehyde, vanillin, 4-methoxybenzoic acid, hexadecanoic acid and z-14-nonacosene. The increased levels of furfural, furfuryl alcohol and anisyl alcohol contribute to the characteristic floral aroma of vanilla tahitensis.

In Table 5.4-6, cells highlighted in green represent compounds reported in vanilla beans for the first time including: acetone, 2-methylpropanal, 4-methyl-2-pentanone, 2-ethenyl-5-methyl-furan, 2(5H)-furanone, 2-hydroxycyclopent-2-en-1-one, 1-methoxy-4-methyl benzene, 3-methylphenol, 3,5-dihydroxy-2-methylpyran-4-one, 4-methoxybenzenacetic acid, lilial acid, kaurene, 1-octadecanol, ethyl heptadecanoate, 9-octadecanoic acid-(z)-methyl ester, z-12-pentacosene and z-14 nonacosene. Table 5.4-6a lists each of the new compounds along with a sampling of previously reported foods and fruits where the compounds were also identified according to the Volatile Components of Foods (VCF) on-line database.

Figure 5.4-6: Total Ion Chromatogram of IFF Tahitian vanilla bean by TDU/DTD GC-MS

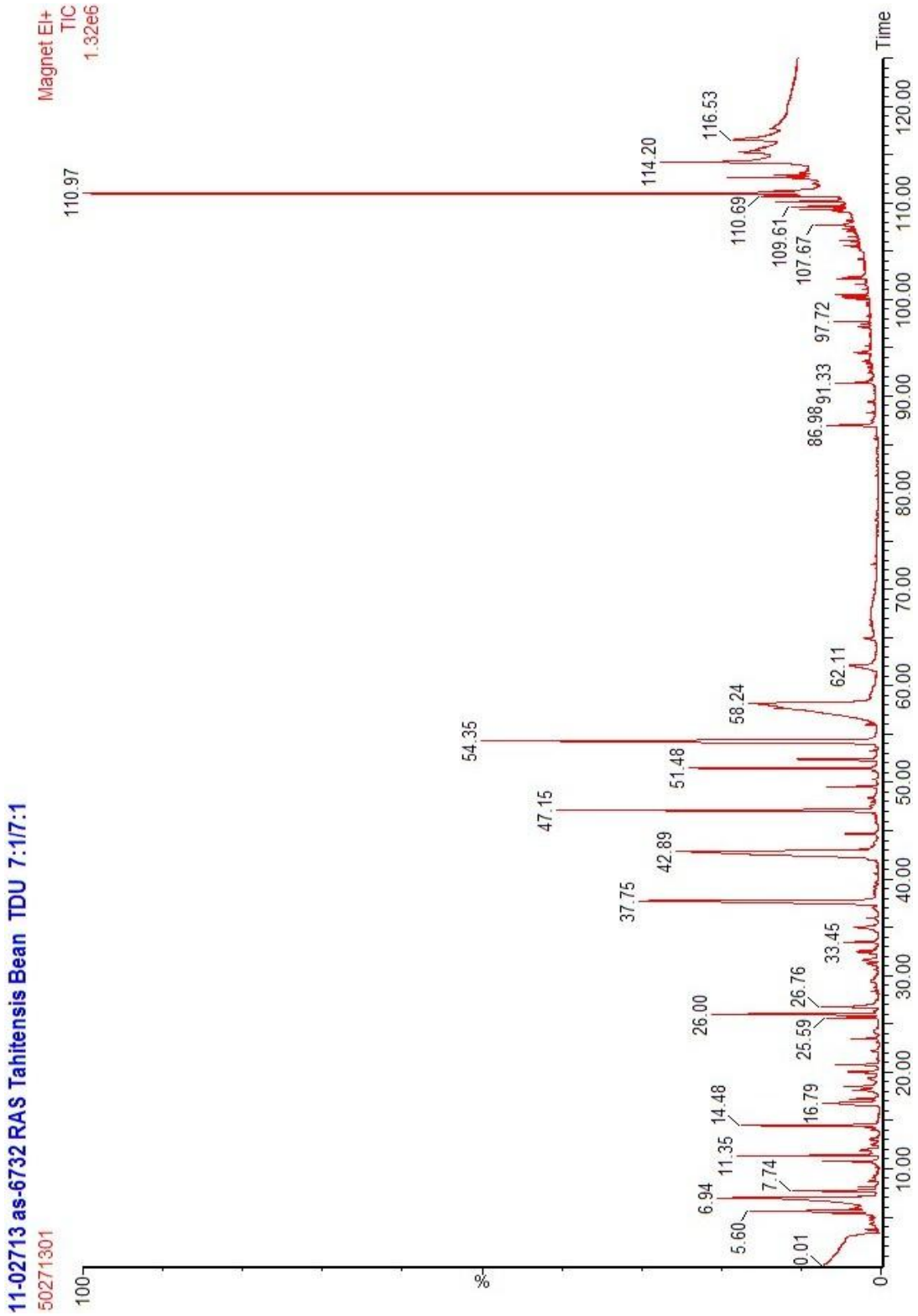


Table 5.4-6: Volatile and semi-volatile compounds identified in IFF Tahitian vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
3.68	acetaldehyde	ethanal	75-07-0	74
4.15	**acetone**		**67-64-1**	19
4.96	**2-methylpropanal**	**iso butyl aldehyde**	**78-84-2**	20
5.39	butane-2,3-dione	diacetyl	431-03-8	288
6.96	acetic acid	ethanoic acid	64.19-7	4122
7.10	2-pentanone	ethyl acetone	107-87-9	trace
8.15	pentane-2,3-dione	acetyl propionyl	600-14-6	84
10.17	**4-methyl-2-pentanone**	**isobutyl methyl ketone**	**108-10-1**	17
10.77	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	525
11.36	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	1338
13.10	hexanal	hexanaldehdye	66-25-1	88
14.48	furan-2-carbaldehyde	furfural	98-01-1	1621
15.40	**2-ethenyl-5-methyl-furan**	**2-methyl-5-vinyl furan**	**10504-13-9**	trace
16.80	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	1205
17.23	cyclopent-4-ene-1,3-dione		930-60-9	279
18.50	**2(5H)-furanone**		**497-23-4**	304
20.79	**2-hydroxycyclopent-2-en-1-one**		**10493-98-8**	424
23.46	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	289
25.99	phenol	phenyl alcohol, benzenol	108-95-2	1980
28.78	**1-methoxy-4-methyl benzene**	**para-methyl anisole**	**104-93-8**	27
31.22	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	177
32.35	methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	611-13-2	200
32.73	**3-methylphenol**	**meta-cresol**	**108-39-4**	16
33.45	2-methoxyphenol	guaiacol	9009-62-5	402
34.59	3-hydroxy-2-methylpyran-4-one	maltol	118-71-8	36
37.71	2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one	hydroxydihydromaltol	8564-83-2	5805
40.55	**3,5-dihydroxy-2-methylpyran-4-one**	**5-hydroxymaltol**	**1073-96-7**	32
41.25	2-methoxy-4-methylphenol	creosol	93-51-6	trace
42.06	1,2-dihydroxybenzene	catechol, pyrocatechol	120-80-9	107
42.85	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	8862
43.14	4-ethenylphenol	p-vinyl phenol	2628-17-3	296
44.66	4-methoxybenzaldehyde	anisaldehyde	123-11-5	330
47.13	(4-methoxyphenyl) methanol	anisyl alcohol	105-13-5	4689
49.55	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	442
50.33	**4-methoxybenzeneacetic acid**	**p-methoxyphenylacetic acid**	**104-01-8**	44
51.17	4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	623-05-2	72
51.47	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000

52.38	4-hydroxybenzaldehyde		123-08-0	1076
53.29	methyl 4-methoxybenzoate	methyl anisate	121-98-2	72
54.32	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	9273
55.96	(4-methoxyphenyl)methyl acetate	anisyl acetate	104-21-2	81
56.23	4-methoxybenzoic acid	p-anisic acid	100-09-4	2122
64.91	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	246
66.26	dodecanoic acid	lauric acid	143-07-7	241
66.52	diethyl phthalate		84-66-2	80
72.59	**4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid**	**lilial acid**	**66735-04-4**	75
77.20	tetradecanoic acid	myristic acid	544-63-8	trace
81.78	6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	502-69-2	17
85.56	methyl hexadecanoate	methyl palmitate	112-39-0	22
86.96	hexadecanoic acid	palmitic acid	57-10-3	675
88.30	ethyl hexadecanoate	ethyl palmitate	628-97-7	55
90.20	**kaurene**		**34424-57-2**	trace
91.33	**1-octadecanol**	**stearyl alcohol**	**112-92-5**	270
91.48	methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	112-63-0	146
91.65	**ethyl heptadecanoate**	**heptadecanoic acid ethyl ester**	**14010-23-2**	trace
91.76	**9-octadecanoic acid (Z)-, methyl ester**		**112-62-9**	45
93.65	octadecanoic acid	stearic acid	57-11-4	135
95.20	docosane		629-97-0	28
97.03	9-tricosene		27519-02-4	49
97.73	tricosane		638-67-5	183
100.0	tetracosane		646-31-1	56
101.6	**z-12-pentacosene**			75
102.4	pentacosane		629-99-2	166
104.2	hexacosane		630-01-3	33
109.3	**z-14 nonacosene**		**54863-80-8**	540
109.6	nonacosane		630-03-5	377

Table 5.4-6a: Newly identified volatile and semi-volatile compounds identified in IFF Tahitian vanilla bean by TDU/DTD GC-MS along with previous identifications as noted in the Volatile Components of Food database.

Compound	Previously Reported
acetone	apples (Mattheis, 1991) starfruit (MacLeod, 1990) chinese quince (Mihara, 1987)
2-methyl propanal	black tea (Bondarovich, 1967) cocoa (Van Praag, 1968) mushrooms (Vidal, 1986) baked potato (Coleman, 1981)
4-methyl-2-pentanone	black tea (Mick, 1984) Chinese quince (Mihara, 1987) cognac (Ledauphin, 2004) bourbon vanilla (Klimes, 1976)
2-ethenyl-5-methyl-furan	coffee (Shimoda, 1990) malt (Farley, 1980)
2(5H)-furanone	roasted filberts (Kinlin, 1972) roasted peanuts (Ho, 1982)
2-hydroxy-2-cyclopenten-1-one	Not found in VCF
1-methoxy-4-methyl benzene	starfruit (MacLeod, 1990)
3-methyl phenol	cherimoya (Idstein, 1984) rambutan (Ong, 1998) roasted filberts (Kinlin, 1972)
3,5-dihydroxy-2-methylpyran-4-one	honey (Shimoda, 1996)
4-methoxybenzeneacetic acid	Not found in VCF
4-(1,1-dimethylethyl)-alpha-methyl benzenepropanoic acid	Not found in VCF
kaurene	cranberry (Anjou, 1967)
1-octadecanol	cherimoya (Idstein, 1984) cranberry (Croteau, 1968) guava (Idstein, 1985)
ethyl heptadecanoate	grilled beef (Hsu, 1982) cognac (Ledauphin, 2004)
9-octadecanoic acid, methyl ester	chive (Hashimoto, 1983) pineapple (Berger, 1985) pear (Creveling, 1970)
z-12-pentacosene	Not found in VCF
z-14 nonacosene	Not found in VCF

5.5 Tanzanian Bean – Bakto Flavors – *V. planifolia*

A sample of Tanzanian vanilla beans was donated by Bakto flavors for analysis. At first examination, the beans appear to be a little less flexible than traditional Bourbon beans. The outside of the beans have a frosting on vanillin on the skin and they have a very rich and creamy vanilla smell. At the time of this dissertation, there are no literature references for the analytical investigation of Tanzanian vanilla beans.

Tanzania has been producing vanilla beans for at least 5 years. However the crop is small and given its geographic proximity to Uganda, the beans are sold through their well-known neighbor. Ugandan vanilla beans are gaining a good reputation as being high quality. With this reputation, it makes sense for Tanzania to sells its vanilla beans through Uganda to get the premium pricing (Fehr, 2010).

As we have seen in previous sections, each analytical technique will tell a different part of the story with regards to compounds that contribute to the aroma impact of the vanilla bean. Since there are no reported analyses of Tanzanian vanilla beans, they will be analyzed by SPME, HSSE, Purge and Trap, and DTD/TDU methods. Further, since all analysis will produce newly identified compounds for Tanzanian vanilla beans, the lines will not be highlighted as in previous sections. The identified packaging contaminants will be highlighted in yellow.

The first analysis was SPME-GC/MS. This was performed with a three-phase fiber to evaluate a wide range of polarities. Recall that this technique is an equilibrium based analysis and highly volatile materials will be prevalent in the results. Figure 5.5-1 is the total ion chromatogram for the SPME-GC/MS analysis of the Tanzanian vanilla bean. The list of identified compounds is shown in Table 5.5-1. A total of 40 compounds were identified in this analysis. There were two contaminants present in the analysis and are highlighted in yellow. One contaminant is limonene which is ubiquitous in concentrating headspace analysis that are performed in a fragrance house. The second contaminant was diethyl phthalate which could have come from the packaging of the beans. The origin of the contaminant is not important, but it should be noted that it is not native to the vanilla bean.

The amounts of each compound in Table 5.5-1 are reported as % area of the chromatogram. As expected, increased amounts of acetic acid, furfural and hexanal were present due to their relative volatility. The vanillin amount is reported as 52.09% of the total area of the chromatogram. This is most likely due to the vanillin frosting on the outside of the beans which makes vanillin very accessible to the headspace above the beans.

The second analysis was HSSE-GC/MS. This analysis was done with a poly dimethyl siloxane coated stir bar. The increased amount of PDMS as compared to the SPME will reveal volatile and semi-volatile non-polar compounds in the headspace. Figure 5.6-2 is the total ion chromatogram for the HSSE-GC/MS analysis of the

Figure 5.5-1: Total Ion Chromatogram of Bakto Flavors Tanzanian vanilla bean by SPME-GC-MS

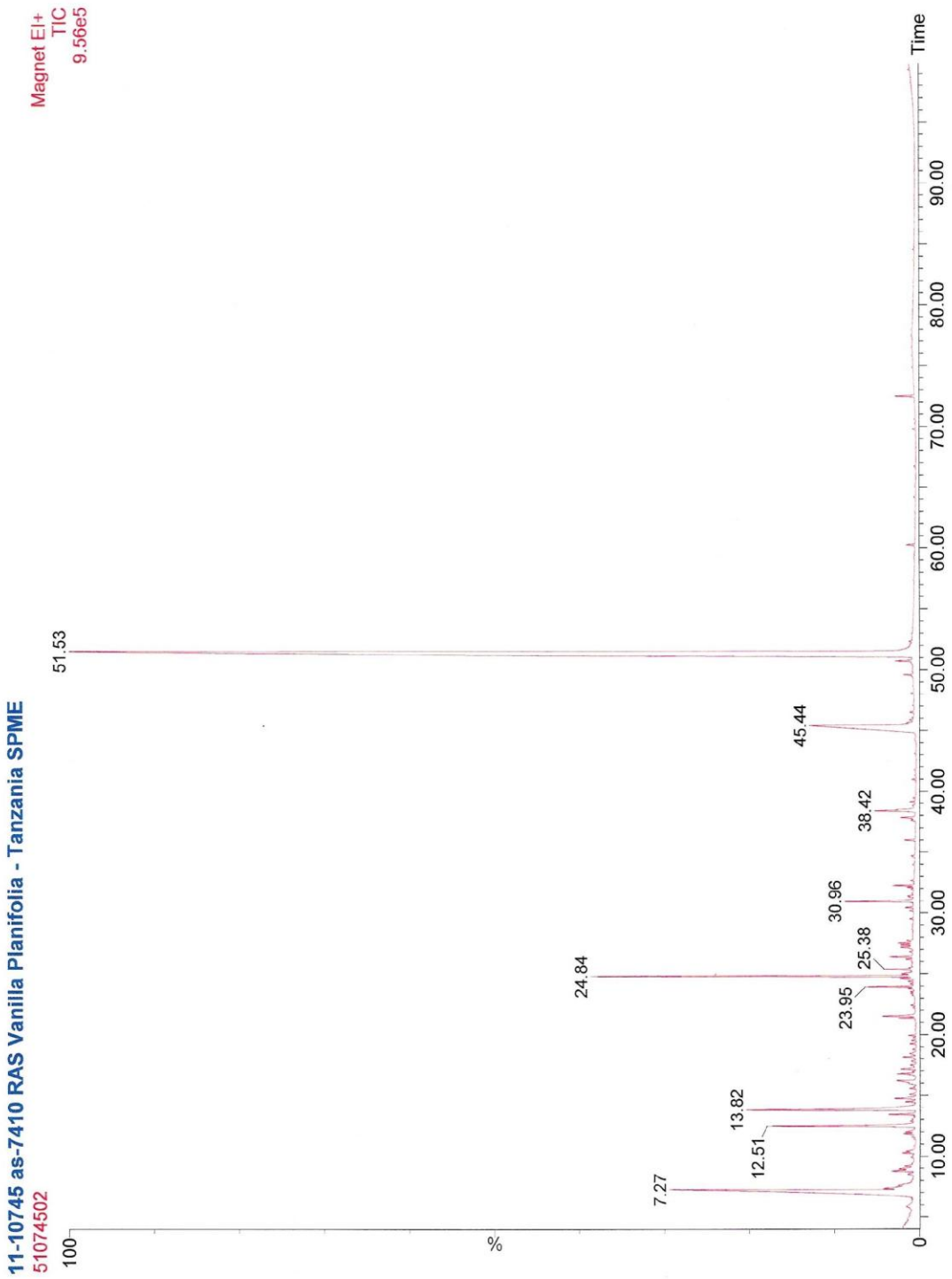


Table 5.5-1: Volatile and semi-volatile compounds identified in Bakto Flavors Tanzanian vanilla bean by SPME-GC-MS.

Time	Assignment	Common name	Registry #	area %
7.21	acetic acid	ethanoic acid	64-19-7	12.84
7.63	3-methylbutanal	Isovaleraldehyde	590-86-3	0.73
8.53	pentanal		110-62-3	0.13
8.81	3-hydroxy-2-butanone	acetal methyl carbinol	513-86-0	0.44
8.97	2-ethyl furan		3208-16-0	0.38
10.30	3-methyl-1-butanol	iso amyl alcohol	123-51-3	0.22
11.40	methylbenzene	toluene	108-88-3	trace
12.02	2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	24347-58-8	0.22
12.52	hexanal	hexanaldehdye	66-25-1	3.49
13.45	octane		111-65-9	0.44
13.83	furan-2-carbaldehyde	furfural	98-01-1	4.38
14.48	1,3-octadiene		1002-33-1	0.15
15.11	2-hexenal		6728-26-3	0.12
15.75	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	trace
19.95	methyl hexanoate	hexanoic acid, methyl ester	106-70-7	0.10
21.56	benzaldehyde		100-52-7	0.75
23.96	1-octen-3-ol	amyl vinyl carbinol	3391-86-4	0.73
24.84	2-pentyl furan		3777-69-3	5.25
27.21	benzyl alcohol	phenylmethanol	100-51-6	0.29
27.40	3-octen-2-one		1669-44-9	0.21
27.55	2-ethyl-1-hexanol		104-76-7	0.25
27.75	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	0.12
30.46	1-octanol	capryl alcohol	111-87-5	0.13
30.96	2-methoxyphenol	guaiacol	9009-62-5	1.21
31.39	2-nonanone	heptyl methyl ketone	821-55-6	0.07
32.10	ethyl heptanoate	heptanoic acid, ethyl ester	106-30-9	trace
32.26	nonanal	nonanaldehyde	124-19-6	0.43
32.68	phenyl ethyl alcohol		60-12-8	0.07
36.02	benzyl acetate	acetic acid, phenylmethyl ester	140-11-4	0.19
38.42	2-methoxy-4-methylphenol	creosol	93-51-6	1.14
39.14	ethyl octanoate	octanoic acid, ethyl ester	106-32-1	0.09
39.50	decanal		112-31-2	trace
41.00	methyl nonanoate	nonanoic acid, methyl ester	1731-84-6	0.07
45.40	nonanoic acid	pelargonic acid	112-05-0	7.33
46.53	2-methoxy-4-vinyl phenol	p-vinylguaiacol	7786-61-0	0.06

50.73	methyl (*E*)-3-Phenylprop-2-enoate	methyl cinnamate	1754-62-7	0.33
51.49	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	52.09
52.34	ethyl decanoate	decanoic acid, ethyl ester	110-38-3	0.06
63.00	diethyl phthalate		84-66-2	trace
72.52	benzyl benzoate		120-51-4	0.35

Tanzanian vanilla bean. The list of identified compounds is shown in Table 5.5-2.

A total of 27 compounds were identified in the HSSE-GC/MS analysis. Two contaminants were present in the analysis. One was the ubiquitous compound limonene and the other was diethylhexyl phthalate which most likely came from the packaging. Other compounds that have been noted as contaminants in this analysis are: dodecanoic acid, tetradecanoic acid, and hexadecanoic acid. Although these are not typical packaging migrants, they are curiously present in a non-heated headspace experiment. The vapor pressures of these compounds would all but eliminate them as being authentic to the vanilla bean in this type of headspace collection. The amounts of each compound in Table 5.5-2 are reported as % area of the chromatogram. As expected, increased amounts of acetic acid, furfural and hexanal were present. The vanillin amount is reported as 46.37% of the total area of the chromatogram. Again, this most likely due to the vanillin frosting on the outside of the vanilla beans being more available to the headspace.

The last of the traditional headspace techniques was purge and trap. This analysis is not biased in terms of polarity as the first two were. SPME is biased not only by equilibrium to the headspace, but also by the compounds affinity to the adsorbent phase. The HSSE method uses a single phase PDMS which lends it to equilibrium bias as well as bias into a non-polar adsorbent. The purge and trap method uses Tenax TA which does not bias by polarity. This technique gives a true picture of the concentrated

headspace above the beans. However, since it is a concentrating technique, there inevitably will be a slight bias to headspace replenishment through volatility.

Figure 5.5-3 is the total ion chromatogram for the Purge and Trap-GC/MS analysis of the Tanzanian vanilla bean. A total of 31 compounds were identified in the Purge and Trap-GC/MS analysis. Two contaminants were present in the analysis. One was the ubiquitous compound limonene and the other was diethyl phthalate which most likely came from the packaging. The amounts of each compound in Table 5.5-3 are reported as % area of the chromatogram. As expected, increased amounts of acetic acid and furfural were present although hexanal was present at a lower amount. Additionally, (1-methylethenyl)-cyclopentane was present at 2.54% which was at a level similar to furfural. The vanillin amount is reported as 12.32%. This is significantly lower than the SPME and HSSE results and demonstrates the affinity bias for compounds like vanillin into a non-polar adsorbent. Again, the high level of vanillin is most likely due to the vanillin frosting on the outside of the vanilla beans.

The last, and perhaps most definitive, experiment was the DTD/TDU-GC/MS analysis. Unlike the previous concentrating headspace experiments, the DTD/TDU method does not bias by polarity or volatility. Further, the use of an internal standard in this desorption method allows the quantitation of identified compounds. Figure 5.5-5 is the total ion chromatograms for the Tahitian vanilla bean. The list of identified compounds is shown in Table 5.5-4. A total of 62 compounds were identified in this analysis. There were three packaging contaminants present in the analysis and they are

Figure 5.5-2: Total Ion Chromatogram of Bakto Flavors Tanzanian vanilla bean by HSSE-GC-MS

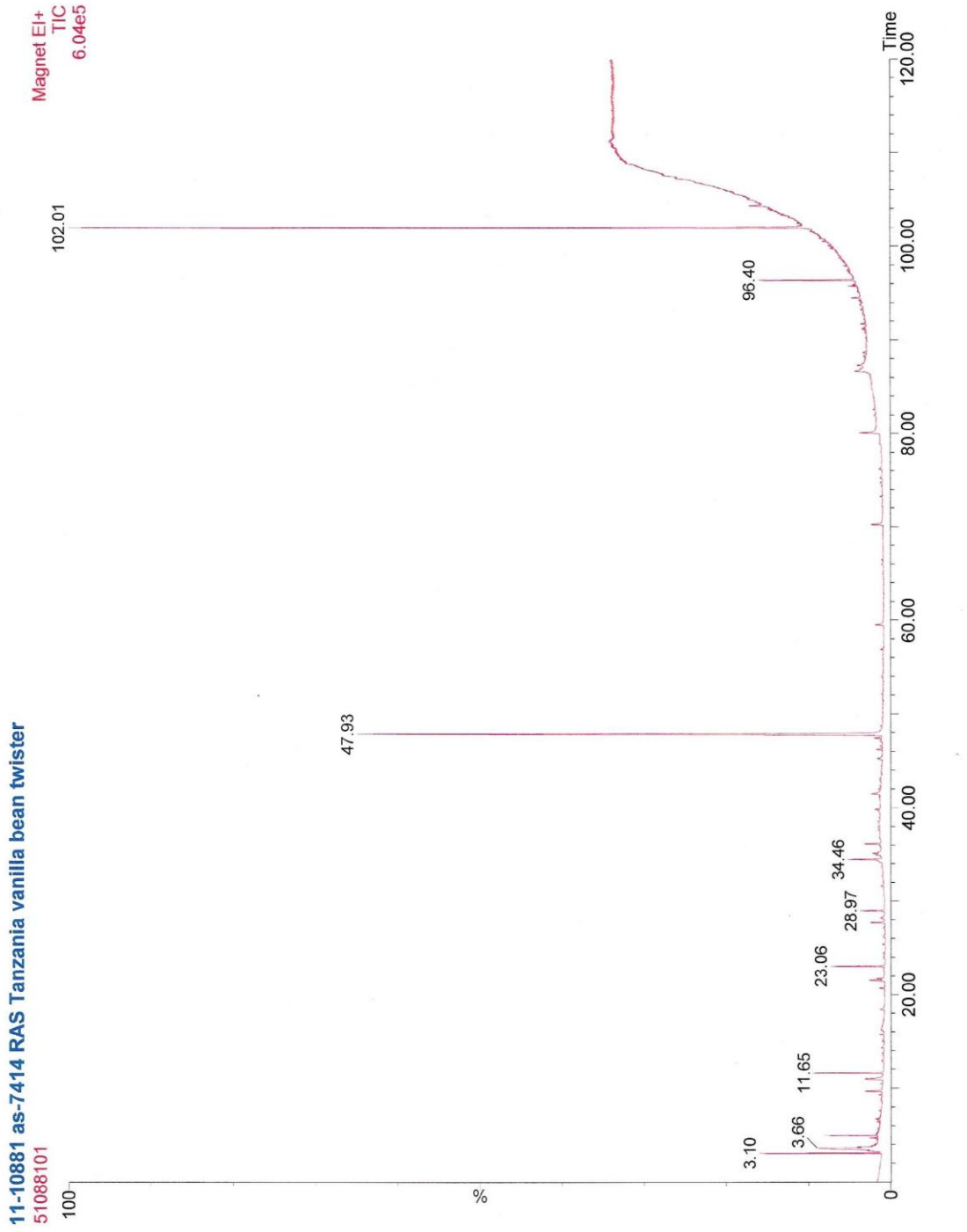


Table 5.5-2: Volatile and semi-volatile compounds identified in Bakto Flavors Tanzanian vanilla bean by HSSE-GC-MS

Time	Assignment	Common name	Registry #	area %
4.73	hexane		110-54-3	0.22
5.00	acetic acid	ethanoic acid	64-19-7	1.93
6.77	heptane		142-82-5	0.38
8.75	methylbenzene	toluene	108-88-3	trace
9.35	2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	24347-58-8	0.15
9.72	hexanal	hexanaldehdye	66-25-1	1.06
11.01	furan-2-carbaldehyde	furfural	98-01-1	2.11
12.93	3-methyl butanoic acid	isovaleric acid	503-74-2	0.13
14.33	2(3H)-furanone, dihydro	gamma-butyrolactone	96-48-0	0.22
15.76	2-butoxy ethanol		111-76-2	0.27
18.44	benzaldehyde		100-52-7	0.41
20.73	1-octen-3-ol	amyl vinyl carbinol	3391-86-4	0.37
21.58	2-pentyl furan		3777-69-3	1.18
21.76	octanal	capryl aldehyde	124-13-0	0.50
24.49	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	0.14
27.71	2-methoxyphenol	guaiacol	9009-62-5	1.08
28.97	nonanal		124-19-6	1.59
31.60	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		28564-83-2	trace
34.98	octanoic acid	caprylic acid	124-07-2	0.50
35.13	2-methoxy-4-methylphenol	creosol	93-51-6	0.74
41.54	nonanoic acid	pelargonic acid	112-05-0	0.75
47.47	2-propenoic acid, 3-phenyl, methyl ester		103-26-4	0.54
47.91	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	46.37
59.70	dodecanoic acid	lauric acid	143-07-7	0.15
70.27	tetradecanoic acid	myristic acid	544-63-8	1.06
80.09	hexadecanoic acid	palmitic acid	57-10-3	3.10
96.40	diethylhexyl phthalate		117-81-7	4.29

Figure 5.5-3: Total Ion Chromatogram of Bakto Flavors Tanzanian vanilla bean by Dynamic Headspace-GC-MS

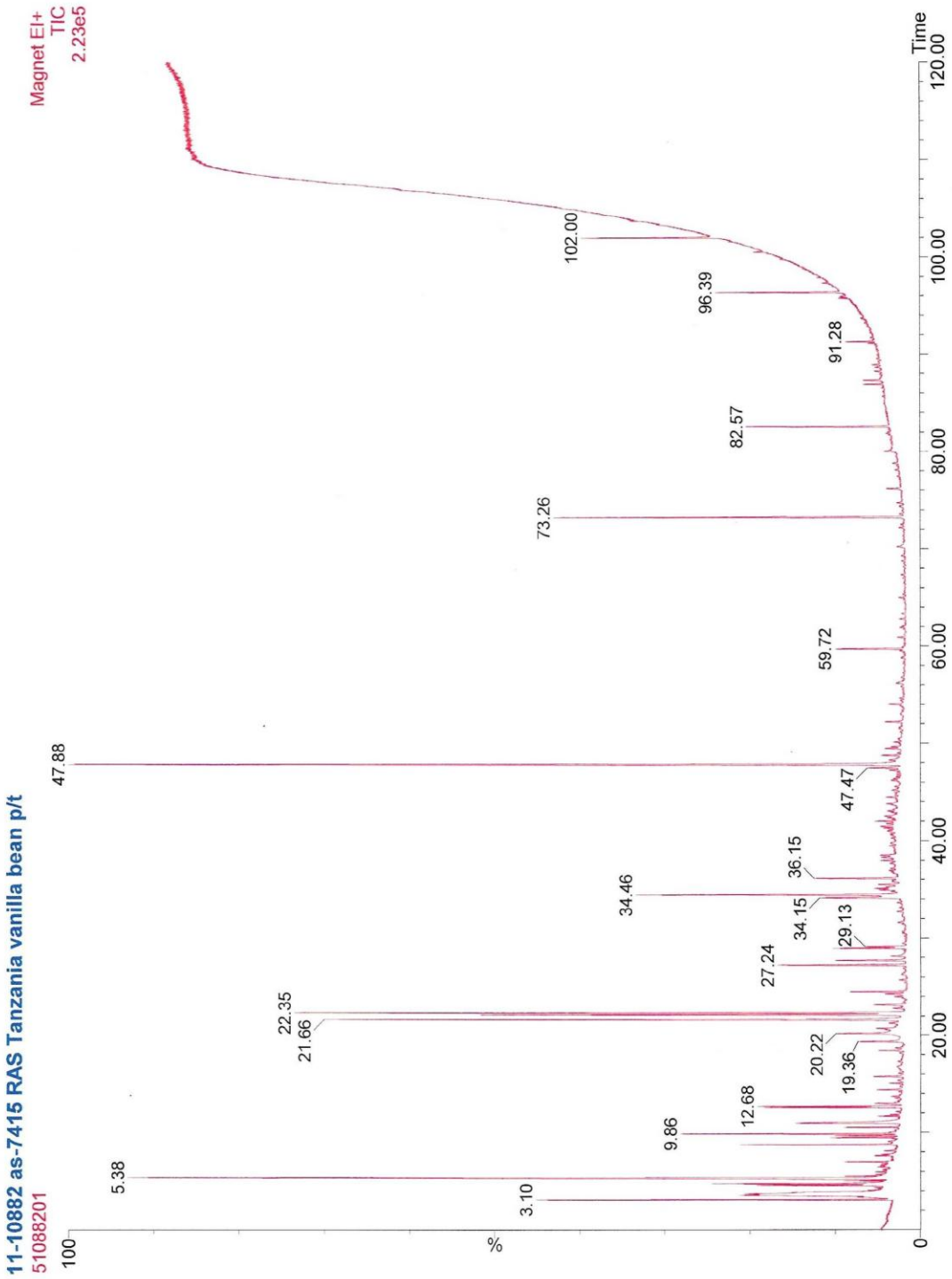


Table 5.5-3: Volatile and semi-volatile compounds identified in Bakto Flavors Tanzanian vanilla bean by Purge & Trap GC-MS.

Time	Assignment	Common name	Registry #	area %
4.75	ethyl acetate		141-78-6	2.06
5.05	oxolane	tetrahydrofuran	109-99-9	0.15
5.34	acetic acid	ethanoic acid	64-19-7	11.04
5.57	2-methyl butanal		96-17-3	0.33
6.99	3-hydroxy-2-butanone	acetoin	513-86-0	0.34
7.64	3-methyl-1-butanol	iso amyl alcohol	123-51-3	0.24
8.77	methylbenzene	toluene	108-88-3	1.35
9.50	1,3-butanediol	b-butylene glycol	107-88-0	0.52
9.70	hexanal	hexanaldehdye	66-25-1	0.35
10.54	octane		111-65-9	0.47
10.98	furan-2-carbaldehyde	furfural	98-01-1	2.20
12.57	(1-methylethenyl)-cyclopentane		55661-02-4	2.54
12.96	3-methyl butanoic acid	isovaleric acid	503-74-2	0.45
13.53	2-methyl butanoic acid	2-methyl butyric acid	116-53-0	0.09
14.39	2(3H)-furanone, dihydro	gamma-butyrolactone	96-48-0	0.29
15.77	2-butoxy ethanol		111-76-2	0.46
18.44	benzaldehyde		100-52-7	0.44
18.73	3-methyl-2(5H)-furanone		22122-36-7	0.12
24.50	1-methyl-4-(1-methylethenyl)-cyclohexene	limonene	5989-27-5	0.68
25.73	1-phenylethanone	acetophenone	96-86-2	0.11
27.24	8,9-dihydrodicylopentadiene			1.69
27.72	2-methoxyphenol	guaiacol	9009-62-5	0.87
28.98	nonanal		124-19-6	0.87
29.13	tricyclo[5.2.1(2,6)]decane		6004-38-2	0.51
29.47	phenyl ethyl alcohol		60-12-8	0.06
36.15	decanal		112-31-2	1.05
47.88	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	12.32
48.75	dodecane, 2,6,10-trimethyl		3891-98-3	0.25
59.72	diethyl phthalate		84-66-2	0.91
73.26	isopropyl myristate		110-27-0	4.24
82.58	isopropyl palmitate		142-91-6	1.53

highlighted in yellow. Since the DTD/TDU method is not a concentrating method, it is not surprising to see limonene was not detected in this analysis.

The analysis revealed high amounts of acetic acid, 3-methylbutanal, 3-methyl pentanal, furfuryl alcohol, 2-hydroxy-2-cyclopenten-1-one, guaiacol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, dimethyl maleic anhydride 3,4-dimethylfuran-2,5-dione, hydroxymethyl furfural, 4-ethenyl-2-methoxyphenol, 4-hydroxybenzaldehyde, palmitic acid, linoleic acid, tricosane and squalene. Remarkably, the vanillin content was the highest of any bean tested in this series of experiments at 3.04%. Although this level of vanillin appears to be extremely high, it does not fall outside of the acceptable range of component ratios established for authentication of vanilla beans. The IOFI (International Organization of the Flavor Industry) has adopted the French government's specifications entitled DGCCRF (Directorate General for Competition, Consumption, and Fraud Repression) which states that certain marker compounds in vanilla beans should be found in certain ratios when authentic. There are established ranges for each compound ratio. Using the ratio of vanillin/4-hydroxybenzaldehyde, the accepted ratio is 10-20. The "acceptable" Bakto Flavors sample ratio is 10.82, the Ugandan sample is 14.52 and the Tanzanian sample is 19.05 respectfully. Even though the overall amount of vanillin appears to be high, the ratio analysis falls within acceptable limits. Certain vanilla beans, especially those from India, were found to be higher than the DGCCRF guidelines (John, 2004). Traditionally a high ratio of vanillin/4-hydroxybenzaldehyde was considered a sign of adulteration caused by immersion of the vanilla bean into an aqueous vanillin solution to improve the concentration of vanillin in the bean. As the solution evaporated,

it would leave behind a coating of vanillin on the outer surface of the bean. In the case of the Tanzanian and Indian beans, it appears that the water within the beans diffuses out and carries the vanillin to the surface of the bean. Once the water evaporates, it leaves behind the coating of vanillin. Another possibility is the sublimation of vanillin. Vanillin can go directly from the solid state to the gaseous state, diffuse through the outer surface of the bean and redeposit on the outer surface as a crystalline solid. In either case, the crystallization of vanillin on the outer surface happens in situ and is not a result of adulteration in these two types of vanilla beans. Figure 5-5.4 is a photograph taken of the surface of the Tanzanian vanilla bean. The photo visibly shows the vanillin crystals on the surface without magnification.

The compounds identified in the Tanzanian vanilla bean are strikingly similar to those identified in the Ugandan bean and the Bourbon bean. Table 5.5-5 shows the results of the most prevalent compounds of each of these three beans that were analyzed by DTD/TDU-GC/MS. This side-by-side comparison shows how similar the beans really are. For most compounds in the comparison, all three are very similar. The Tanzanian bean lacks furfural and hydroxymethylfurfural compared to the other two. Further, the compounds identified in the Tanzanian vanilla bean have been previously reported in other analysis, either within the focus of this work, or within the literature review table in Section 2.

Although the analysis performed on the Tanzanian bean shows it to be very similar to the Ugandan, and therefore Bourbon variants, it should be noted the

experimentation was only done on one lot of beans. Replicate studies of varying lots should be done before firm conclusions can be drawn on the quality and similarities of Tanzanian vanilla beans.

Figure 5-5.4: Unmagnified photograph of Tanzanian vanilla bean showing the vanillin crystals on the surface

Source: Photograph taken by the author.

Figure 5.5-5: Total Ion Chromatogram of Bakto Flavors Tanzanian vanilla bean by TDU/DTD GC-MS

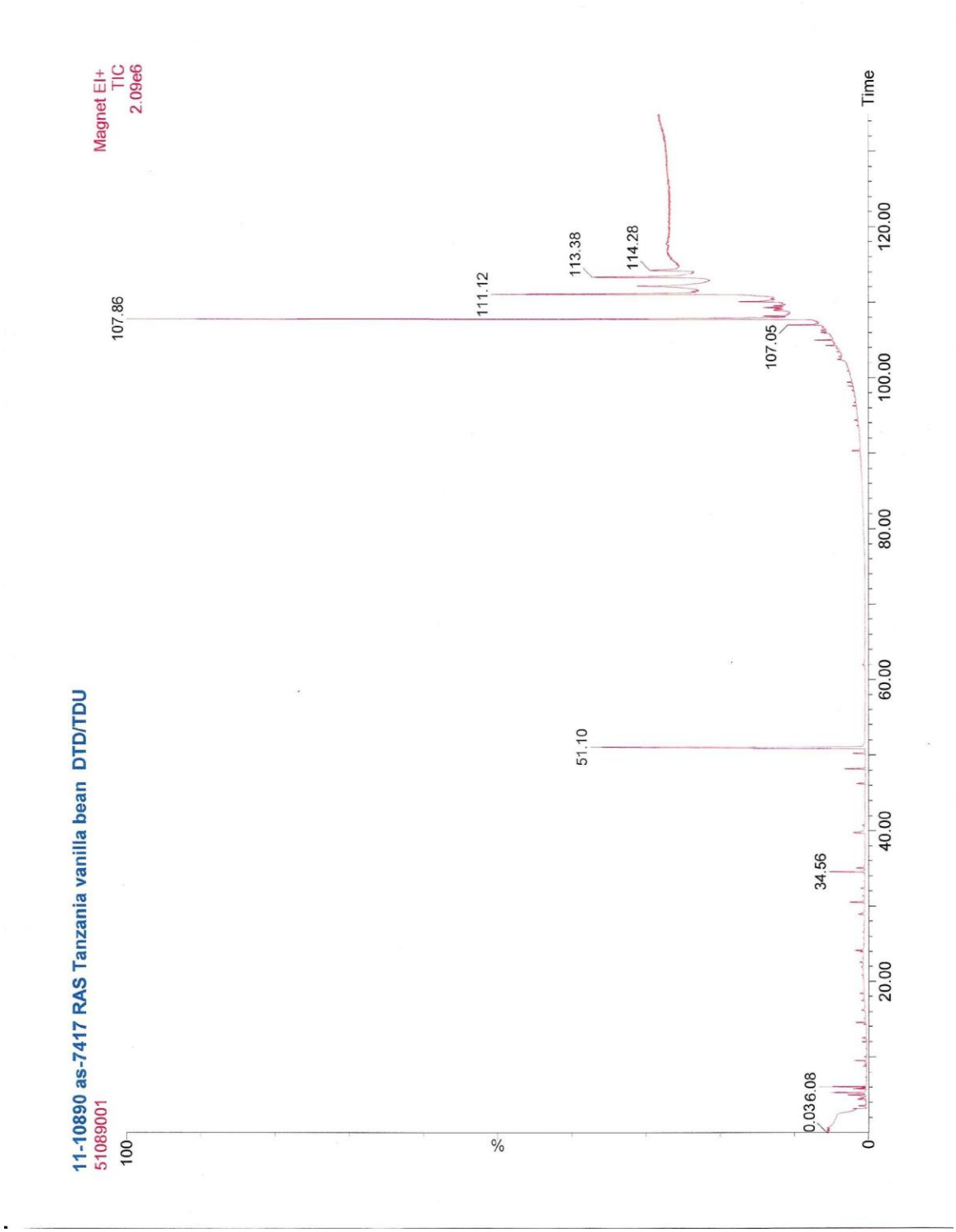


Table 5.5-4: Total Ion Chromatogram of Bakto Flavors Tanzanian vanilla bean by TDU/DTD GC-MS

Time	Assignment	Common Name	Registry #	ppm (w/w)
4.5	butane-2,3-dione	diacetyl	431-03-8	75
5.3	acetic acid	ethanoic acid	64-19-7	3764
5.8	3-methylbutanal	Isovaleraldehyde	590-86-3	681
6.8	pentane-2,3-dione	acetyl propionyl	600-14-6	trace
9.2	3-methylpentan-2-one	sec-butyl-methyl ketone	565-61-7	trace
9.5	3-methylpentanal	3-methyl valeraldehyde	15877-57-3	1157
12.5	furan-2-carbaldehyde	furfural	98-01-1	356
13.3	furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	98-00-0	820
14.6	2(3H)-furanone, dihydro	gammabutyrolactone	96-48-0	377
15.1	cyclopent-4-ene-1,3-dione	4-cyclopentene-1,3-dione	930-60-9	197
18.7	2-hydroxy-2-cyclopenten-1-one		10493-98-8	561
20.4	3-methyl-2-oxopentanoic acid	methyl ethyl pyruvic acid	1460-34-0	66
20.8	5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	620-02-0	172
21.0	2,3,5-hexanetrione, 4-hydroxy		4384-08-1	trace
22.6	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one		10230-62-3	399
24.0	phenol	phenyl alcohol, benzenol	108-95-2	341
25.6	2-hydroxy-3-methyl-2-cyclopentene-1-one		80-71-7	30
27.4	4-hydroxy-5-methyl-3(2H)-furanone		19322-27-1	46
29.1	4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	3658-77-3	147
29.9	2-hydroxy-1-(2-furyl)-ethanone		17678-19-2	trace
30.5	2-methoxyphenol	guaiacol	9009-62-5	730
34.5	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		28564-83-2	4326
35.1	3,4-dimethylfuran-2,5-dione	dimethyl maleic anhydride	766-39-2	989
39.1	5-hydroxymaltol		1073-96-7	81
39.3	2-methoxy-4-methylphenol		93-51-6	trace
39.8	5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	67-47-0	2659
40.7	4-ethenylphenol	4-vinyl phenol	2628-17-3	206
46.3	4-ethenyl-2-methoxyphenol	4-Hydroxy-3-methoxystyrene	7786-61-0	891
48.2	2,6-dimethoxyphenol	SYRINGOL (INTERNAL STANDARD)	91-10-1	2000
50.2	4-hydroxybenzlaldehyde		123-08-0	1595
51.1	4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	30396
54.3	4-methoxybenzoic acid	p-anisic acid	100-09-4	136

54.6	4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol	498-00-0	37
55.9	4-hydroxy-benzoic acid, methyl ester	methyl paraben	99-76-3	37
56.5	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	498-02-2	21
58.5	methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	3943-74-6	27
60.1	1-(4-hydroxy-3-methoxyphenyl)-2-propanone	vanillyl methyl ketone	2503-46-0	trace
62.0	4-hydroxy-3-methoxybenzoic acid	vanillic acid	121-34-6	350
71.6	3-buten-2-one, 4-(4-hydroxyphenyl)	oxyphenalon	3160-35-8	54
72.1	tetradecanoic acid	myristic acid	544-63-8	180
74.5	4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one		1080-12-2	33
77.4	6,10,14-trimethyl-2-pentadecanone		502-69-2	48
78.5	pentadecanoic acid	pentadecylic acid	1002-84-2	60
83.3	hexadecanoic acid	palmitic acid	57-10-3	781
84.4	hexadecanoic acid, ethyl ester	ethyl palmitate	628-97-7	22
88.8	heneicosane		629-94-7	26
89.6	9,12 octadecadienoic acid	linoleic acid	60-33-3	546
90.2	ethyl 9,12-octadecadienoate	ethyl linoleate	544-35-4	128
91.4	octadecanoic acid, ethyl ester	ethyl stearate	111-61-5	21
91.8	docosane		629-97-0	64
93.8	9-tricosene		27519-02-4	71
94.5	tricosane		638-67-5	502
96.4	hexanedioic acid, bis(2-ethylhexyl)ester	dioctyl adipate	103-23-1	404
96.7	tetracosane		646-31-1	15
97.8	dipropylene glycol dibenzoate		20109-39-1	116
99.0	pentacosane		629-99-2	431
99.5	diethylhexyl phthalate		117-81-7	337
101.0	hexacosane		630-01-3	106
102.9	heptacosane		593-49-7	177
104.3	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	111-02-4	510
105.6	nonacosane		630-03-5	167
108.4	hentriacontane		630-04-6	294

Table 5.5-5: Comparison of major components identified by TDU/DTD GC-MS

Compound	Madagascar Bourbon	Ugandan	Tanzanian
acetic acid	4071	3806	3764
3-methylbutanal	492	579	681
3-methylpentanal	1030	687	1157
furfural	1288	762	356
furfuryl alcohol	882	952	820
phenol	249	273	341
furaneol	59	125	147
guaiacol	729	693	730
hydroxymethylfurfural	8409	5376	2659
4-hydroxybenzaldehyde	1900	1424	1595
vanillin	20940	20692	30396
vanillic acid	437	834	350

5.5.1 Analytical Precision of TDU/DTD GC-MS Method

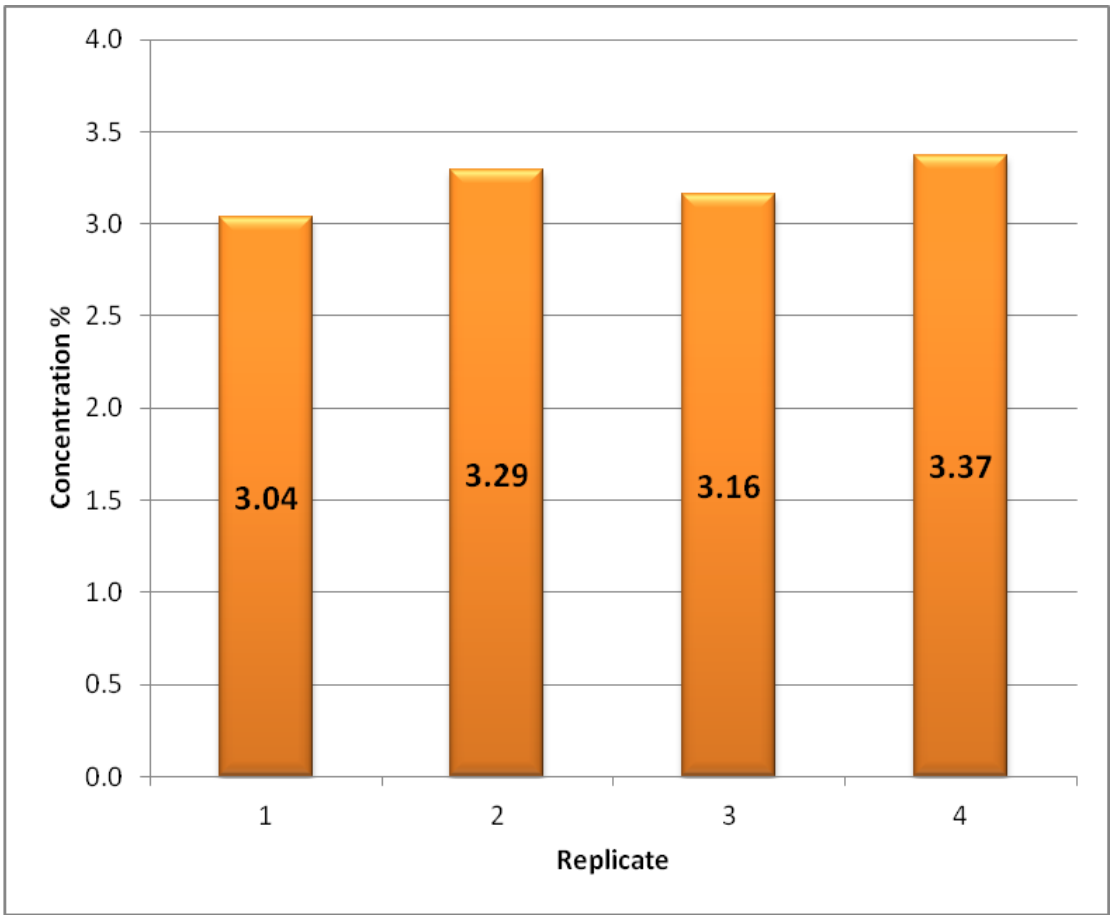
The TDU/DTD GC-MS analysis was repeated three times in addition to the initial experiment to determine the analytical precision of the method. For each experiment, a new composite of vanilla bean and chromsorb was created and thermally desorbed. The results of the replicate study are listed in Table 5.5.1-1. Compounds that were initially identified at trace levels were omitted from the replicates as they possess no statistical meaning with respect to precision. Since each replicate was run with a new composite, the variation in beans is taken into account as well as the homogeneity of the composite. Similar precision studies were conducted by Adedeji in his doctoral dissertation with similar results (Adedeji, 1993b). Most compounds exhibit a relative standard deviation of 10-20%. Figure 5.5.1-1 shows the vanillin concentrations of all four replicates of the Tanzanian vanilla bean. The relative standard deviation for the replicates was only 4.5% and the standard deviation was 0.15. This result is better than expected and is indicative of good analytical precision and reproducibility.

Table 5.5.1-1: Replication of Tanzanian vanilla bean experiment using TDU/DTD-GC-MS for determination of precision of the method

Assignment	Rep #1	Rep #2	Rep #3	Rep #4	%RSD	Average	Standard Deviation
butane-2,3-dione	75	77	59	64	**12.6**	69	8.66
acetic acid	3764	7701	8467	5468	**33.7**	6350	2142.76
3-methylbutanal	681	286	258	218	**59.7**	361	215.53
3-methylpentanal	1157	1507	1645	1300	**15.4**	1402	216.51
furan-2-carbaldehyde	356	550	529	482	**18.2**	479	87.07
furan-2-ylmethanol	820	967	873	721	**12.2**	845	102.74
2(3H)-furanone, dihydro	377	769	609	380	**35.7**	534	190.80
cyclopent-4-ene-1,3-dione	197	205	195	218	**5.1**	204	10.46
2-hydroxy-2-cyclopenten-1-one	561	612	521	542	**7.0**	559	38.93
3-methyl-2-oxopentanoic acid	66	331	334	253	**51.1**	246	125.72
5-methylfuran-2-carbaldehyde	172	196	206	139	**16.7**	178	29.83
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	399	639	614	363	**28.3**	504	142.74
phenol	341	401	376	362	**6.8**	370	25.30
2-hydroxy-3-methyl-2-cyclopentene-1-one	30	44	52	24	**34.1**	38	12.79
4-hydroxy-5-methyl-3(2H)-furanone	46	51	71	33	**31.4**	50	15.78
4-hydroxy-2,5-dimethylfuran-3-one	147	98	131	128	**16.2**	126	20.45
2-methoxyphenol	730	1923	1953	1194	**41.0**	1450	594.61
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	4326	4022	3866	3221	**12.1**	3859	466.19
dimethyl maleic anhydride 3,4-dimethylfuran-2,5-dione	989	967	840	981	**7.4**	944	69.99
5-hydroxymaltol	81	128	151	130	**24.1**	123	29.56
5-(hydroxymethyl)furan-2-carbaldehyde	2659	2590	2182	2490	**8.5**	2480	210.45
4-ethenylphenol	206	279	206	220	**15.2**	228	34.71
4-ethenyl-2-methoxyphenol	891	785	596	519	**24.5**	698	170.69
4-hydroxybenzlaldehyde	1595	1510	1567	1573	**2.3**	1561	36.28
4-hydroxy-3-methoxybenzaldehyde	30396	32869	31588	33713	**4.5**	32141	1455.19
4-methoxybenzoic acid	136	101	127	144	**14.7**	127	18.67
4-(hydroxymethyl)-2-methoxyphenol	37	21	25	34	**25.6**	29	7.50
4-hydroxy-benzoic acid, methyl ester	37	18	32	31	**27.5**	30	8.10

1-(4-hydroxy-3-methoxyphenyl)ethanone	21	15	24	29	**26.3**	22	5.85
methyl 4-hydroxy-3-methoxybenzoate	27	51	29	41	**30.3**	37	11.20
4-hydroxy-3-methoxybenzoic acid	350	153	177	305	**39.0**	246	96.03
3-buten-2-one, 4-(4-hydroxyphenyl)	54	61	49	37	**20.1**	50	10.11
tetradecanoic acid	180	222	237	187	**13.3**	207	27.40
4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one	33	29	22	trace	**19.9**	28	5.57
6,10,14-trimethyl-2-pentadecanone	48	33	41	34	**18.3**	39	7.16
pentadecanoic acid	60	75	71	51	**17.0**	64	10.92
hexadecanoic acid	781	866	808	644	**12.1**	775	94.11
hexadecanoic acid, ethyl ester	22	17	20	trace	**13.6**	20	2.68
heneicosane	26	29	21	24	**13.5**	25	3.39
9,12 octadecadienoic acid	546	681	604	573	**9.7**	601	58.36
ethyl 9,12-octadecadienoate	128	144	159	108	**16.3**	135	21.89
octadecanoic acid, ethyl ester	21	26	19	trace	**16.9**	22	3.68
docosane	64	79	61	55	**15.8**	65	10.22
9-tricosene	71	78	61	49	**19.5**	65	12.61
tricosane	502	588	531	549	**6.6**	542	36.07
tetracosane	15	19	22	trace	**18.9**	19	3.53
pentacosane	431	399	419	374	**6.1**	406	24.82
hexacosane	106	99	125	116	**10.2**	112	11.37
heptacosane	177	148	133	169	**12.7**	157	19.92
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	510	498	338	525	**18.6**	468	87.20
nonacosane	167	111	198	208	**25.5**	171	43.64
hentriacontane	294	229	288	242	**12.4**	263	32.57

Figure 5.5.1-1: Analytical precision data for vanillin in Bakto Flavors Tanzanian vanilla bean by TDU/DTD-GC-MS



5.6 Synthesis Work

5.6.1 Introduction

Recently, a series of vanilla beans were analyzed by Direct Thermal Desorption GC-MS by Lee (2006) which included some cured, wild vanilla beans from a Peruvian rainforest. The species of the bean is not known. These beans were found to contain a series of anisyl alcohol esters that have not been previously described as occurring in vanilla. The compounds anisyl acrylate, anisyl salicylate, anisyl anisate, anisyl vanillate, anisyl protocatechuate, anisyl myristate, anisyl pentadecanoate, anisyl palmitate, anisyl linoleate, anisyl oleate and approximately 10 unidentified anisyl alcohol esters were found. Of this list of novel compounds, three compounds were selected based on cost and availability of starting materials to be synthesized, characterized and evaluated for odor value.

5.6.2 Reaction Mechanism

The reaction mechanism chosen for the synthesis of the novel anisyl alcohol esters was a nucleophilic addition/elimination reaction between acyl chlorides and alcohols. This reaction takes place in the presence of triethylamine which serves to neutralize the hydrogen chloride produces as well as catalyze the reaction. This reaction

mechanism is an easy and direct way of producing an ester from an alcohol because it happens at room temperature, is a relatively quick reaction and is irreversible. An alternative would be making an ester from an alcohol and a carboxylic acid. However, this reaction requires heat, has slow kinetics and is reversible. Acid catalyzed ester hydrolysis reactions require a large excess of alcohol and the removal of evolved water to drive the reaction forward.

The general reaction mechanism for nucleophilic addition/elimination is shown in Figure 5.6-1. In the addition step, the lone pair of electrons on the nucleophile attacks the

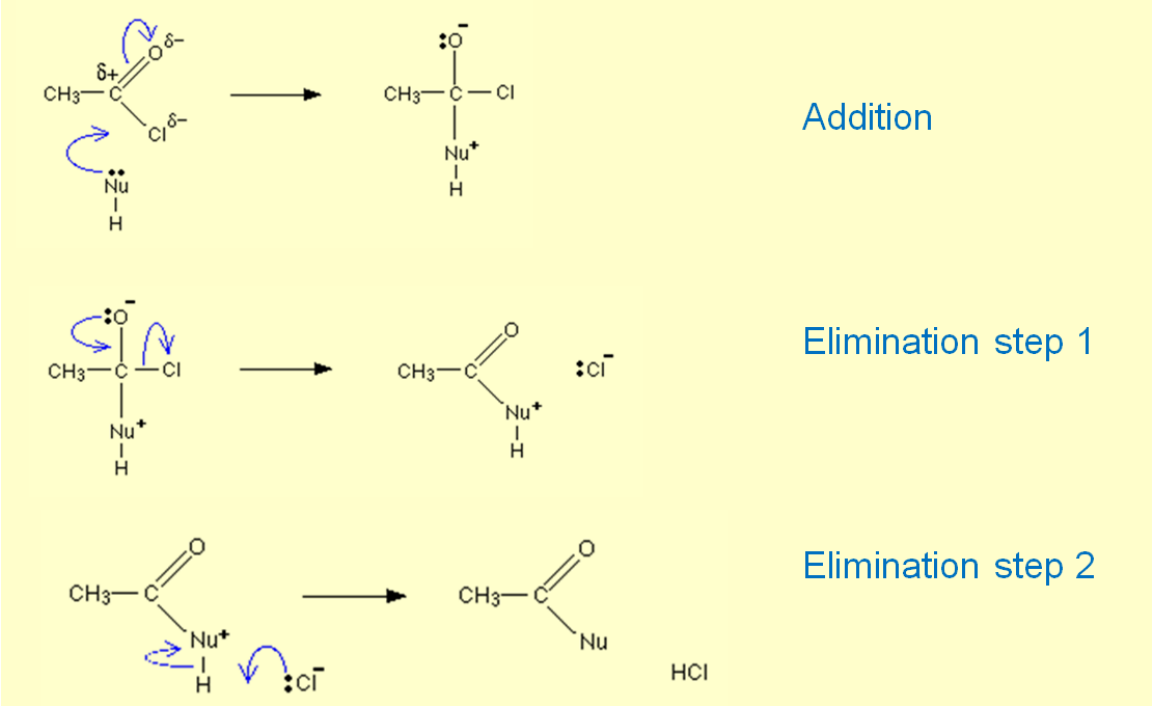


Figure 5.6-1: Reaction mechanism for nucleophilic addition/elimination reaction

Source: www.chemguide.co.uk

partial positive carbon of the polarized carbonyl bond of the acyl chloride. This forms a tetrahedral intermediate which can easily expel good leaving groups. The elimination part of the mechanism occurs in two distinct steps. In the first step, the carbonyl double bond reforms and the electrons in the chlorine-carbon bond are repelled until they reside entirely on the chlorine, forming a chloride ion. In the second step of the elimination mechanism, the chloride ion sequesters the hydrogen off of the original nucleophile as a hydrogen ion, which leaves the electrons behind on the oxygen and cancels the positive charge.

The post reaction wash steps serve very specific purposes. The initial water wash hydrolyzes unreacted acyl chloride (if any remains) to the corresponding acid which is water soluble. The water wash also helps to remove residual HCl from the reaction mixture as well as unreacted p-anisyl alcohol. The second wash with saturated sodium bicarbonate solution helps to neutralize any HCl that might remain, and to further remove p-anisyl alcohol. The last wash with brine serves to remove residual water from the organic reaction layer.

5.6.3 Anisyl Anisate

The reaction between p-anisyl alcohol and p-anisoyl chloride was carried out as described in section 4.1 according to the mechanism is section 5.1.2. The GC-FID analysis of the crude reaction mixture shows a large product peak with an ester index

value of 1896. GC-MSD analysis and NMR confirmed the product of the reaction to be anisyl anisate. Evaluation of the crude reaction mixture by GC-ODP revealed a pleasant sweet cherry note. In order to accurately evaluate the reaction product, the material must be pure. An Isolera Flash Purification unit was used to purify the material and a 1% solution was made of pure product in propylene glycol for evaluation. Several members of the R&D Technical Perfumery Laboratory at International Flavors and Fragrances evaluated the diluted product and gave several descriptors including: cherry, coumaric, heliotropic, jasmonic, sugary and sweet.

5.6.4 Anisyl Myristate

The reaction between p-anisyl alcohol and myristoyl chloride was carried out as described in section 4.1 according to the mechanism is section 5.6.2. The GC-FID analysis of the crude reaction mixture shows a large product peak with an ester index value of 2204. GC-MSD analysis and NMR confirmed the product of the reaction to be anisyl myristate. Evaluation of the crude reaction mixture by GC-ODP revealed no odor for the reaction product.

5.6.5 Anisyl Palmitate

The reaction between p-anisyl alcohol and palmitoyl chloride was carried out as described in section 4.1 according to the mechanism is section 5.1.2. The GC-FID analysis of the crude reaction mixture shows a large product peak with an ester index

value of 2416. GC-MSD analysis and NMR confirmed the product of the reaction to be anisyl myristate. Evaluation of the crude reaction mixture by GC-ODP revealed no odor for the reaction product.

6. Conclusions

6.1 HPLC Method Development

In this series of experiments we have shown that analysis times obtained with a traditional HPLC system, when equipped with the correct plumbing, can greatly benefit from shorter LC columns with smaller diameter particle packing. Although our results were not equivalent to those expected with a UPLC system, we tried to demonstrate what was possible using existing equipment with minimal investment beyond the columns. The initial HPLC analysis time for the vanilla phenolics was reduced from 13.45 minutes to 1.86 minutes. As an additional benefit of the time savings, the usage of acetonitrile was reduced 2.7 times.

6.2 Volatile Analysis Technique Comparison

The methods of solid phase microextraction (SPME), headspace sorptive extraction (HSSE) and traditional dynamic headspace, or purge and trap, were critically compared when analyzing two samples of bourbon vanilla beans. One vanilla bean sample was considered to be a good representation of what is commercially acceptable. The other was a rejected bean that had an alcoholic, fermented off-note of unknown origin to the odor. Several studies have been published on the use of SPME for extraction and analysis of vanilla extracts, but none have been reported on raw vanilla beans or on the use of a three phase mixed bed fiber (DVB/CBXN/PDMS) for the analysis of vanilla. The HSSE technique is relatively new being first reported by Bicchi

et al. in 2000 (Bicchi, *et al.* 2000). Only a few studies have been published on the HSSE technique but none on the analysis of vanilla bean have been reported. Dynamic headspace is an older and more intricate headspace technique, but added value to the series of experiments by demonstrating the adsorption of compounds on Tenax is not selective. The critical comparison technique highlighted differences between the two beans, showcased the differences between the techniques and adsorbent mediums used and in the process, identified 24 new compounds in Bourbon vanilla beans that have not been previously reported in the literature.

6.3 DTD/TDU-GC-MS Method Development

A modified version of the original direct thermal desorption headspace gas chromatography – mass spectrometry analysis, pioneered by Hartman in 1992 and Adedeji in 1993, and later by Lee in 2006 for the analysis of vanilla beans was developed using state of the art instrumentation to achieve improved resolution. The initial method yielded detailed information on volatile and semi-volatile components in raw vanilla beans, but lacked high resolution, especially of the high-boiling materials due to equipment limitations. The new method retains the principles of the original in that the vanilla bean sample is still prepared with an inert chromatographic support and is ballistically heated to introduce the sample into the instrument. The new method uses a cryo-focused gas chromatograph inlet system, instead of a cryo-cooled GC oven to improve resolution. Additionally, newer gas chromatographs use electronic pneumatic control which allows the linear velocity to remain constant, independent of oven

temperature. This ensures maximum resolution at all temperatures throughout the oven program. Lastly, the latest double focusing magnetic sector mass spectrometer was used with extremely fast scan and interscan rates. The increased speed of data acquisition ultimately leads to increased resolution.

6.4 DTD/TDU-GC-MS results for vanilla beans

Several vanilla beans were analyzed using the new DTD/TDU-GC-MS method including both Bakto Flavors Bourbon vanilla beans from the volatile analysis comparison, an additional Bourbon vanilla bean, an Indonesian vanilla bean, a Ugandan vanilla bean and a Tahitian vanilla bean. The new method did provide increased resolution of the peaks later in the chromatogram and 30 new compounds were identified in the samples. Unfortunately, highly detailed EI-GC spectral libraries for high molecular weight compounds are not as well populated as lower molecular weight compounds, which hindered some interpretations and rendered others elusive.

6.5 Tanzanian vanilla bean analysis

Various headspace GC-MS techniques, including SPME, HSSE, P&T and DTD were performed as part of an integrated approach to determine the volatile and semi-volatile components of Tanzanian vanilla beans. The compounds identified in the Tanzanian bean closely resemble those found in Ugandan and Bourbon vanilla beans with the exception of the high concentration of vanillin. Additionally, the Tanzanian

bean was used to determine the analytical precision of the DTD/TDU-GC-MS method which proved to be acceptable.

6.6 Synthesis

Three compounds were selected from a series of anisyl alcohol esters which were identified by Direct Thermal Desorption GC-MS analysis of cured, wild vanilla beans from a Peruvian rainforest by Lee (2006). Anisyl anisate, anisyl myristate and anisyl palmitate were synthesized using nucleophilic addition/elimination reaction between varying acyl chlorides and p-anisyl alcohol. Each compound was fully characterized and evaluated for odor value. Anisyl anisate was purified and evaluated by technical perfumers who gave several descriptors including: cherry, coumaric, heliotropic, jasmonic, sugary and sweet. Anisyl myristate and anisyl palmitate did not have odor value most like due to their long chain length.

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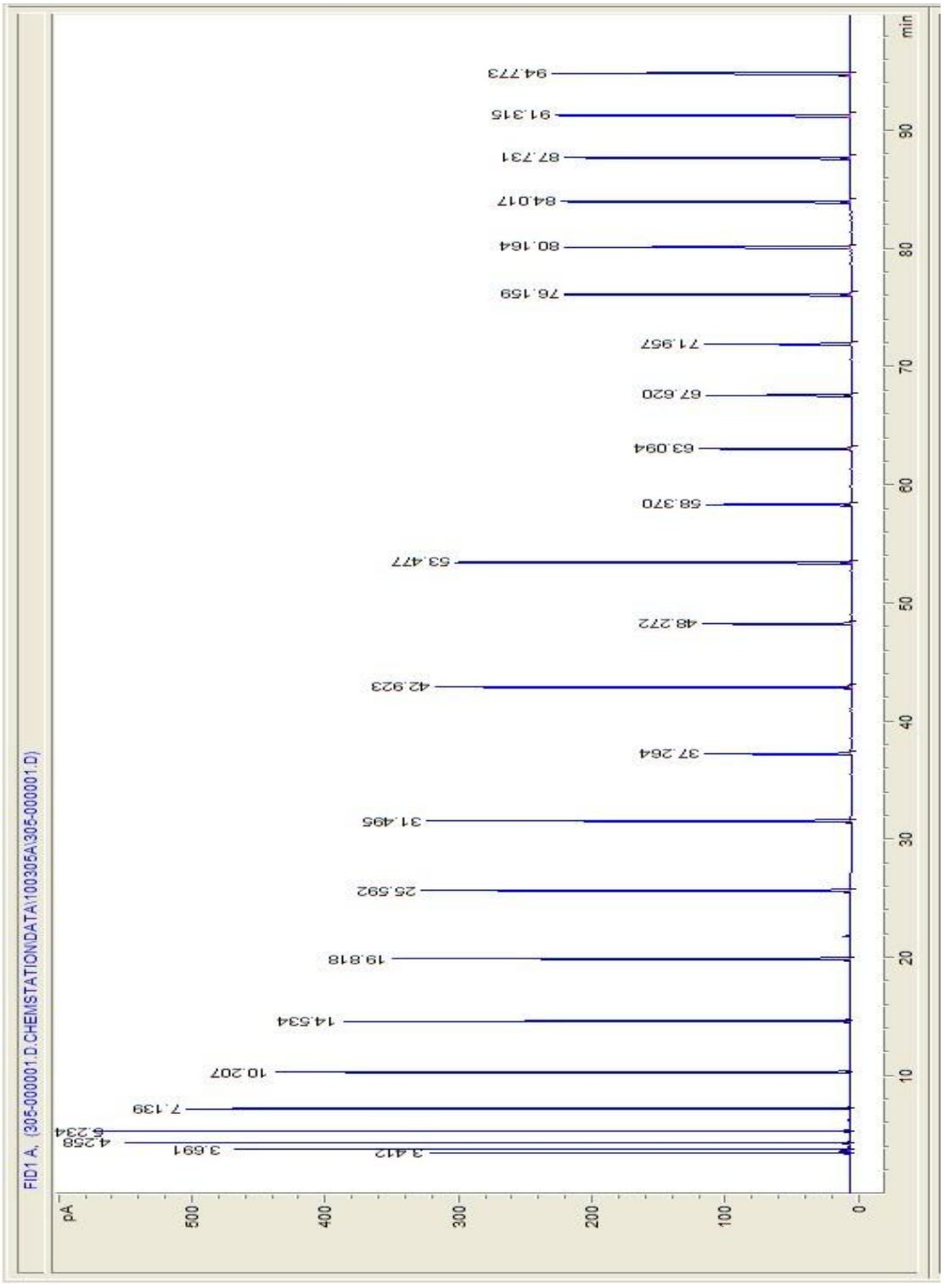
8. Appendicies

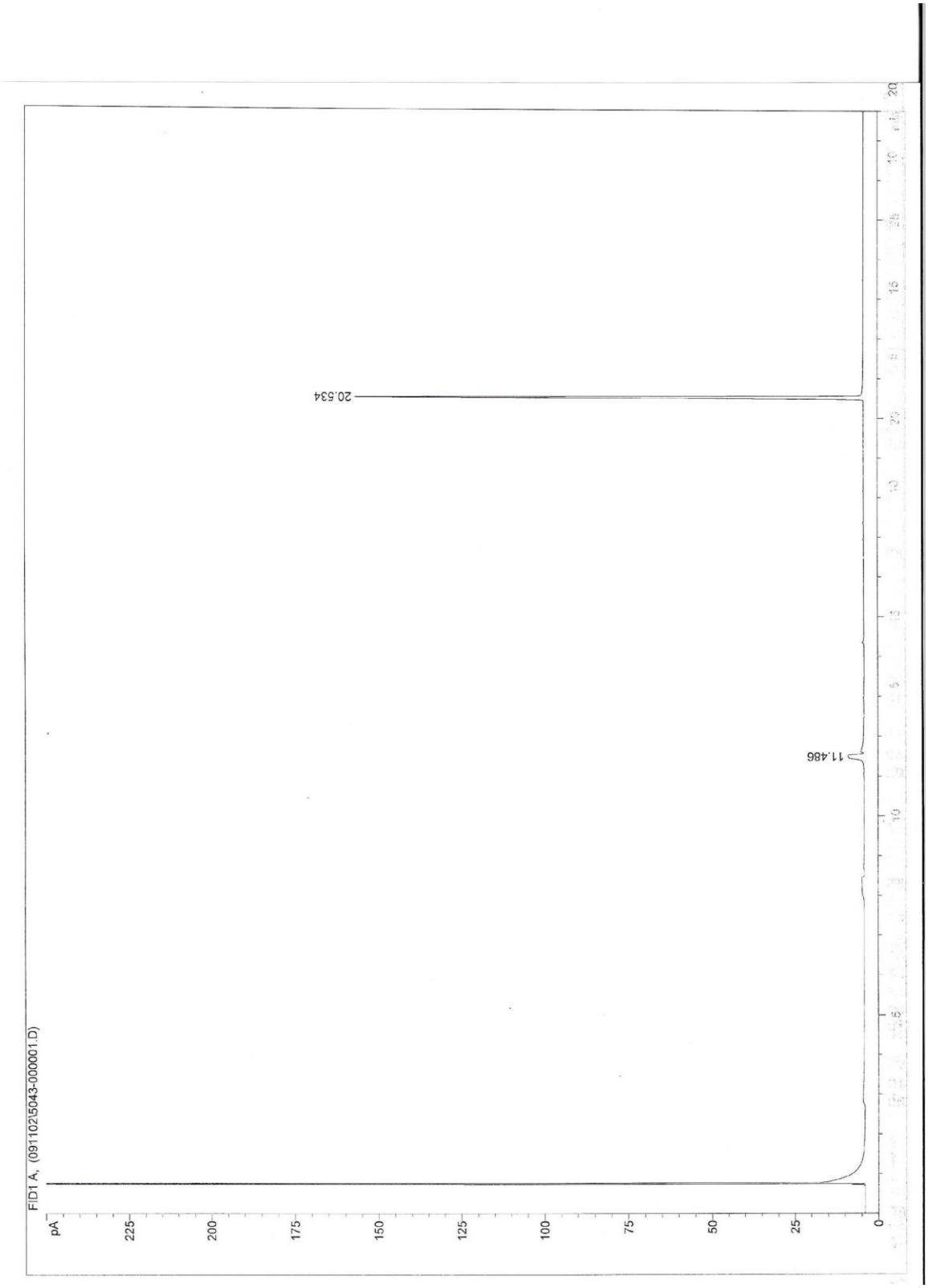
8.1 Ester calibration run for GC-FID



8.2 GC-FID Ester report for Anisyl Anisate

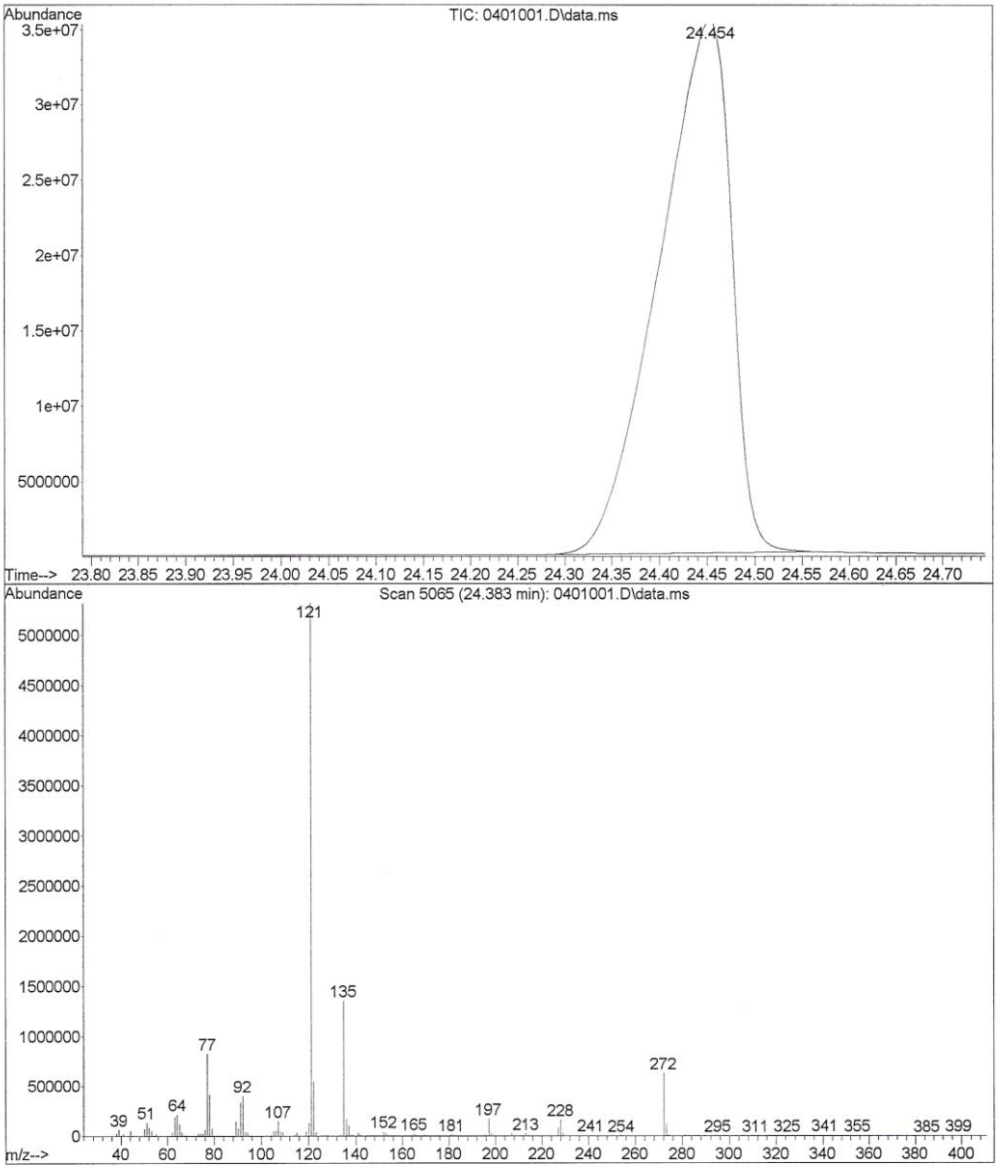
```
Sample Name :  frac 21-24 bulked in dcm                                    Page   1 of 1
Bar Code    :
FID1 A,  (091102\5043-000001.D)

 methyl silicone - 50 m x 0.32 mm x 0.5 um bonded fused silica


Operator         :  GCLAB7-GC5043
Instrument       :  GC5043
Acquired on      :  02-Nov-09, 14:41:39
                        GC CONDITIONS
Initial Temp =   75 Initial Time = 0.00 Inj A Temp =  250 Inj B Temp=  0.00
Rate =  7.59         Det A Temp =   250       Det A Temp =   250
Final Temp (R1) =  225.00
Final Time (R1) =  8.00
Calibration date/time:    Thu Oct 29 08:32:11 2009

Sample Info  :

RET TINDEX CALCULATIONS FOR SIGNAL 1

  RET TIME    INDEX          AREA      AREA%
 11.486      11.926             33     8.094
 20.534      18.964            380    91.906

NUMBER OF PEAKS FOR SIGNAL 1 =  2
TOTAL AREA FOR SIGNAL 1 =  413.869251
```

8.3 GC-FID Chromatogram for Anisyl Anisate

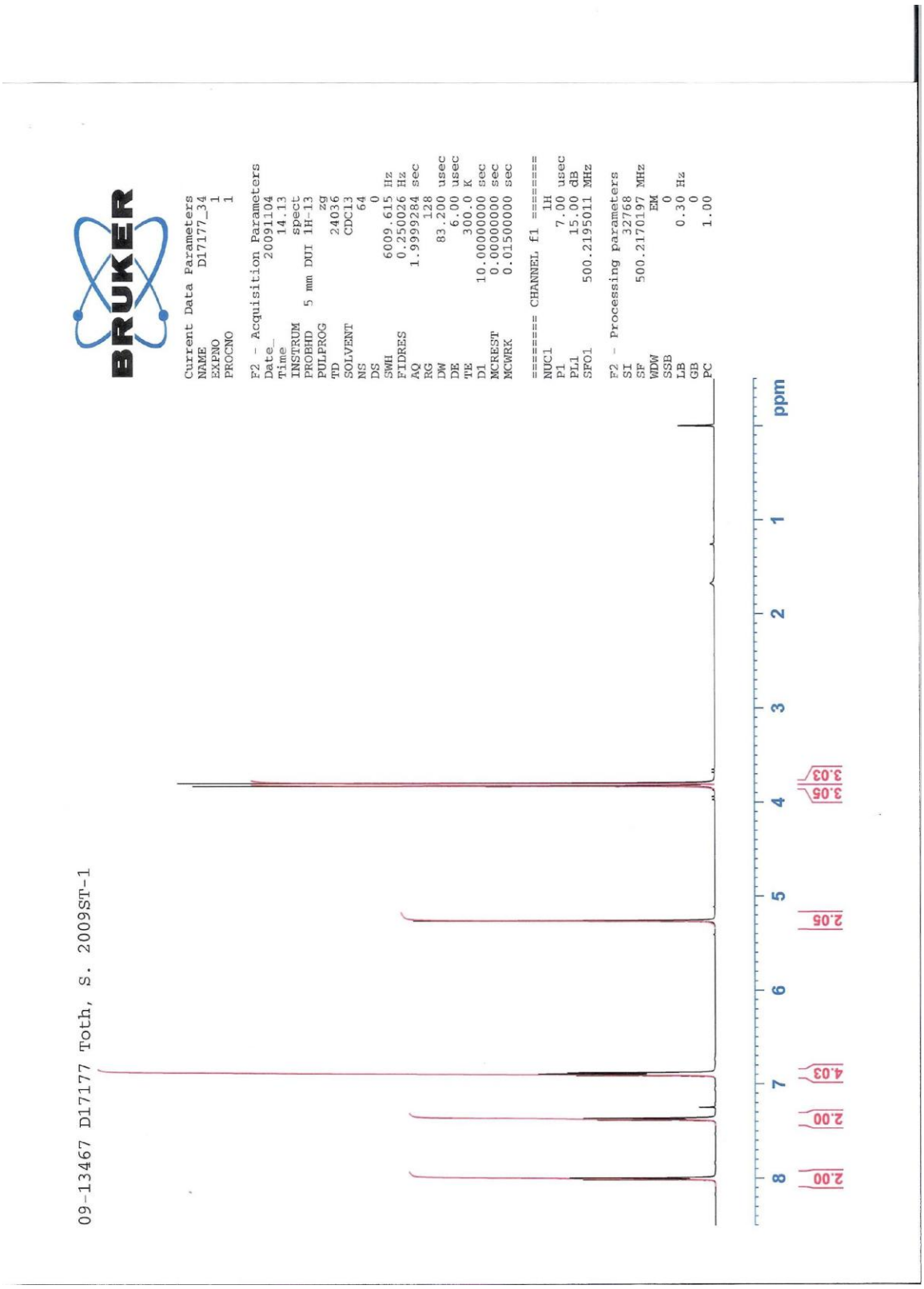


8.4 GC-MSD chromatogram for Anisyl Anisate

```
File       :K:\Data\110209\0401001.D
Operator   : RP
Acquired   :  2 Nov 2009  16:12     using AcqMethod LI Front MSD SCJ OE.M
Instrument :    GCFAS5 - MS
Sample Name: steve's sample
Misc Info  :
Vial Number: 4
```



8.5 NMR spectra for Anisyl Anisate



8.6 GC-FID Ester report for Anisyl Myristate

Sample Name : anisyl myristate Page 1 of 1
Bar Code :
FID1 A, (100121\305-000004.D)

METHYL SILICONE - 50 METER x 0.32 MM x 0.5u

Operator : GCLAB11-GC0305
Instrument : GC0305
Acquired on : 21-Jan-10, 22:49:30

GC CONDITIONS

Initial Temp = 75 Initial Time = 0.00 Inj A Temp = 250 Inj B Temp= 0.00
Rate = 2.00 Det A Temp = 250 Det A Temp = 250
Final Temp (R1) = 315.00
Final Time (R1) = 20.00
Calibration date/time: Thu Jan 21 17:36:53 2010

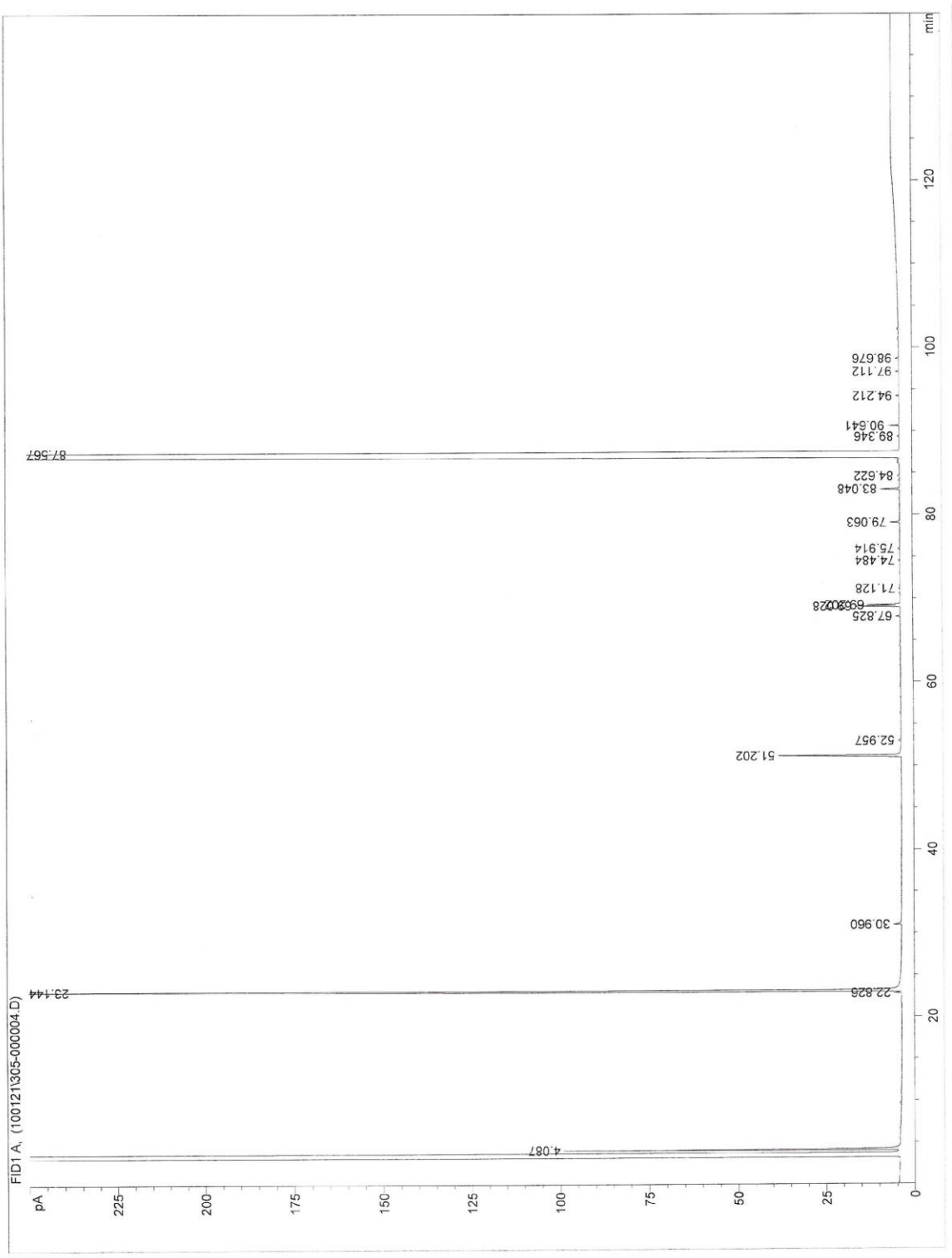
Sample Info :

RET TINDEX CALCULATIONS FOR SIGNAL 1

RET TIME	INDEX	AREA	AREA%
4.087	3.076	497	1.497
22.826	8.614	11	0.032
23.144	8.669	2625	7.899
30.960	9.992	13	0.039
51.202	13.638	283	0.853
52.957	13.974	5	0.014
67.825	17.128	7	0.022
69.028	17.405	73	0.219
69.202	17.445	54	0.161
71.128	17.888	2	0.007
74.484	18.681	3	0.009
75.914	19.022	4	0.011
79.063	19.807	17	0.050
83.048	20.831	32	0.097
84.622	21.247	5	0.015
87.567	22.040	29565	88.955
89.346	22.535	4	0.011
90.641	22.896	19	0.058
94.212	23.923	6	0.017
97.112	24.760	6	0.017
98.676	25.211	6	0.019

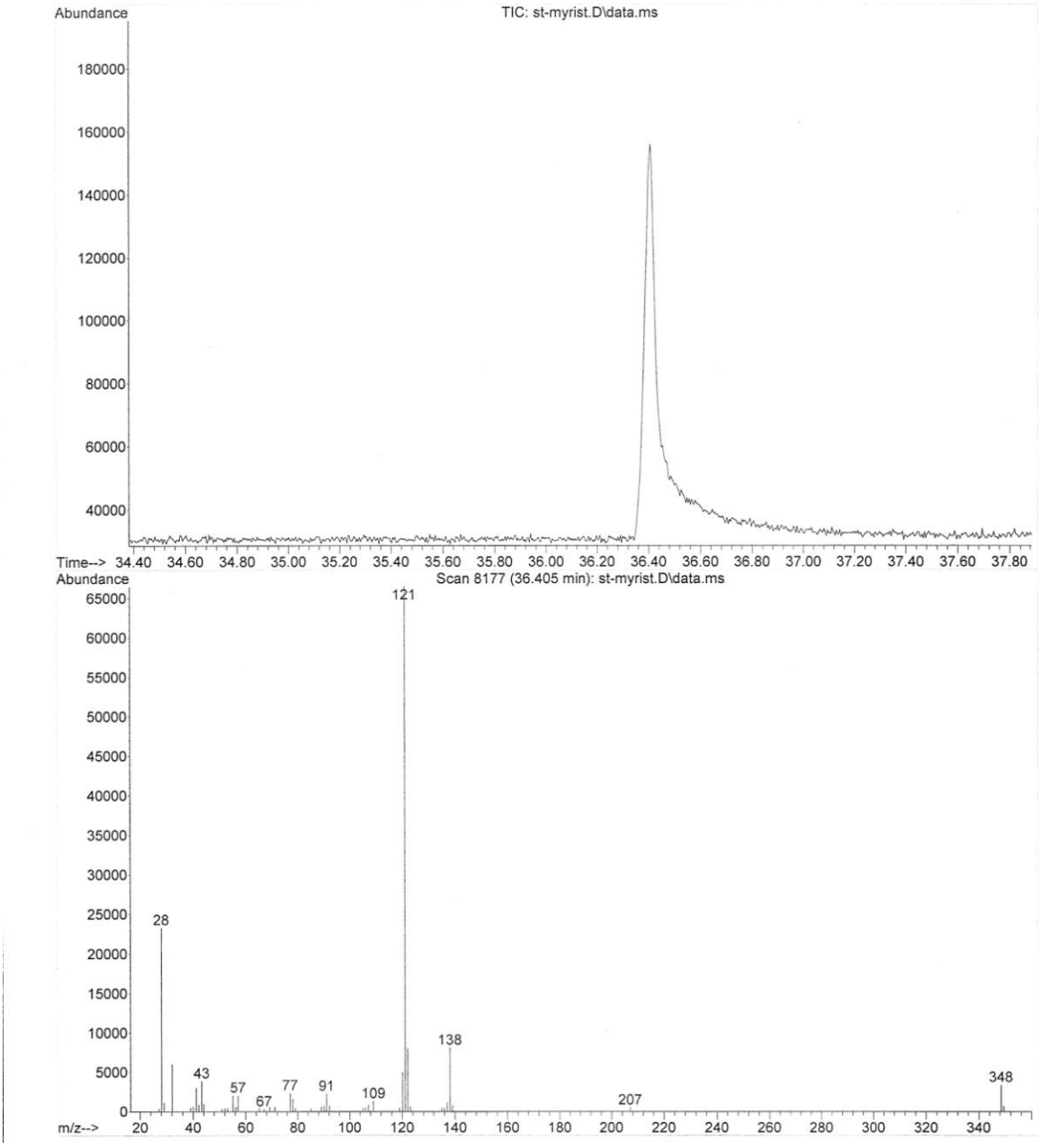
NUMBER OF PEAKS FOR SIGNAL 1 = 21
TOTAL AREA FOR SIGNAL 1 = 33236.4587

8.7 GC-FID Chromatogram for Anisyl Myristate

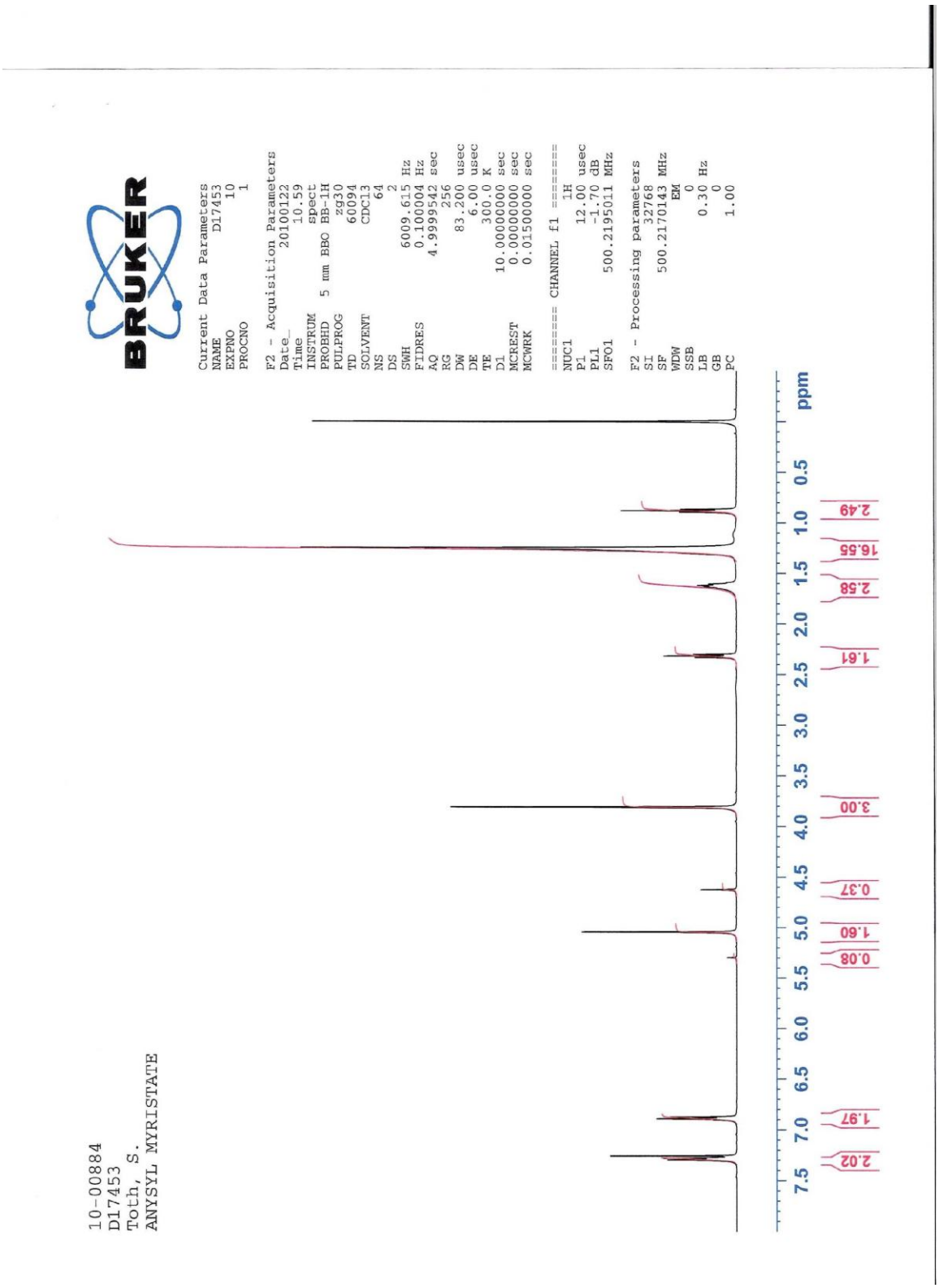


8.8 GC-MSD chromatogram for Anisyl Myristate

```
File       :C:\msdchem\1\DATA\st-myrist.D
Operator   :
Acquired   : 19 Mar 2010  11:55     using AcqMethod EI_FAST320.M
Instrument :    GCMSD3
Sample Name: anisyl myristate
Misc Info  :
Vial Number: 3
```



8.9 NMR spectra for Anisyl Myristate



8.10 GC-FID Ester report for Anisyl Palmitate

Sample Name : anisyl palmitate crude crystalline Page 1 of 2
Bar Code :
FID1 A, (100113\305-000001.D)

METHYL SILICONE - 50 METER x 0.32 MM x 0.5u

Operator : GCLAB11-GC0305
Instrument : GC0305
Acquired on : 13-Jan-10, 11:07:18

GC CONDITIONS

Initial Temp = 75 Initial Time = 0.00 Inj A Temp = 250 Inj B Temp= 0.00
Rate = 2.00 Det A Temp = 250 Det A Temp = 250
Final Temp (R1) = 315.00
Final Time (R1) = 20.00
Calibration date/time: Tue Jan 12 17:53:47 2010

Sample Info :

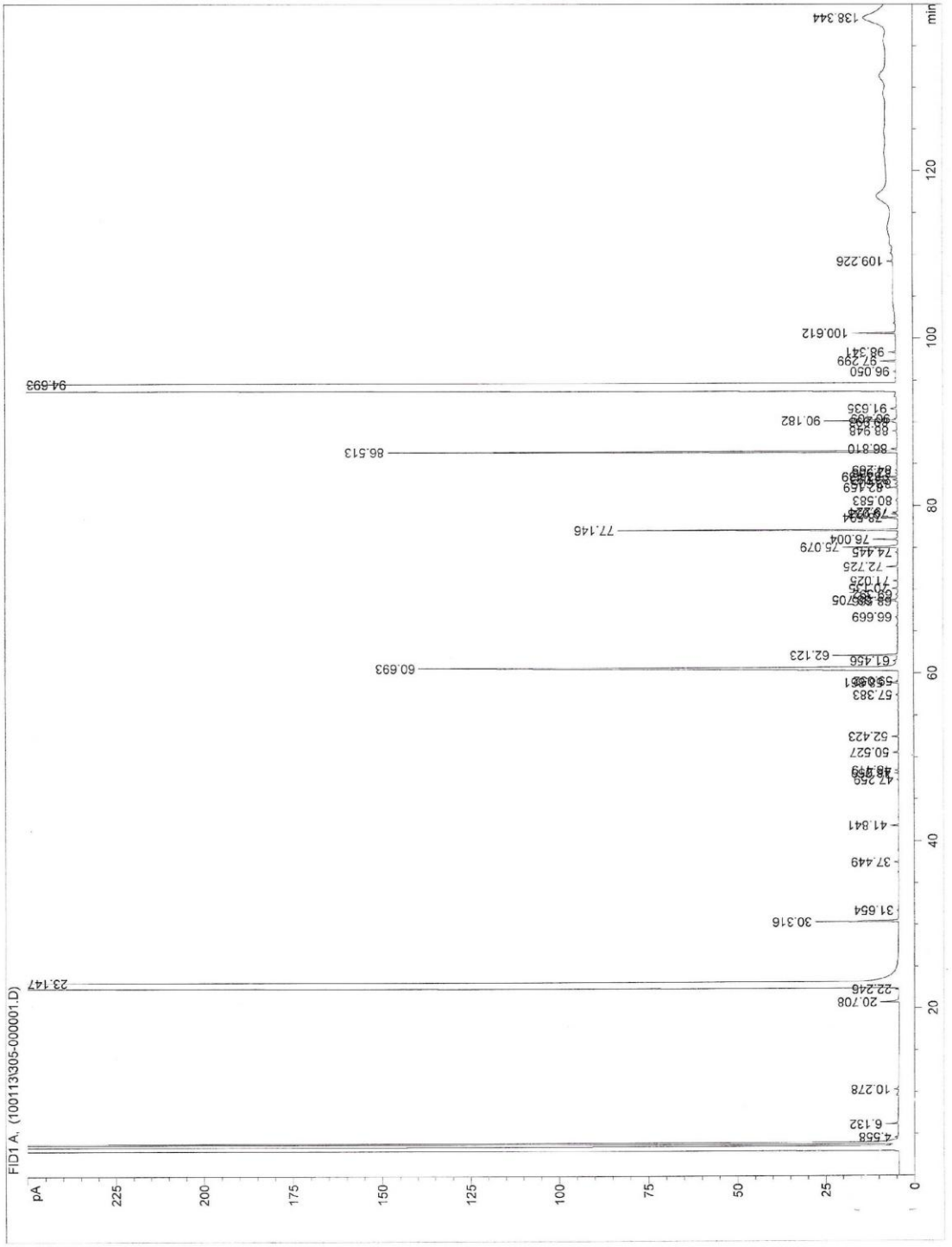
RET TINDEX CALCULATIONS FOR SIGNAL 1

RET TIME	INDEX	AREA	AREA%
4.558	3.739	3	0.003
6.132	4.744	16	0.017
10.278	6.178	7	0.007
20.708	8.306	32	0.034
22.246	8.574	5	0.006
23.147	8.731	32086	33.846
30.316	9.951	159	0.168
31.654	10.182	2	0.003
37.449	11.187	7	0.007
41.841	11.960	12	0.013
47.259	12.967	3	0.003
48.059	13.119	7	0.007
48.419	13.187	6	0.006
50.527	13.590	9	0.010
52.423	13.952	11	0.011
57.383	14.959	4	0.004
58.861	15.269	18	0.019
59.052	15.309	3	0.003
60.693	15.655	1823	1.923
61.456	15.815	5	0.005
62.123	15.956	103	0.109
66.669	16.954	3	0.003
68.566	17.388	3	0.003
68.705	17.419	36	0.038
69.392	17.577	4	0.004
70.135	17.747	9	0.010
71.025	17.951	7	0.008
72.725	18.352	18	0.019
74.445	18.760	4	0.004
75.079	18.910	100	0.106
76.004	19.135	44	0.046
77.146	19.419	473	0.499
78.594	19.779	25	0.026
79.033	19.888	10	0.011
79.224	19.936	9	0.009
80.583	20.285	5	0.005
82.159	20.692	18	0.019
82.605	20.807	4	0.004
83.093	20.933	8	0.008
83.439	21.023	21	0.022
83.903	21.148	4	0.004
84.265	21.245	3	0.003

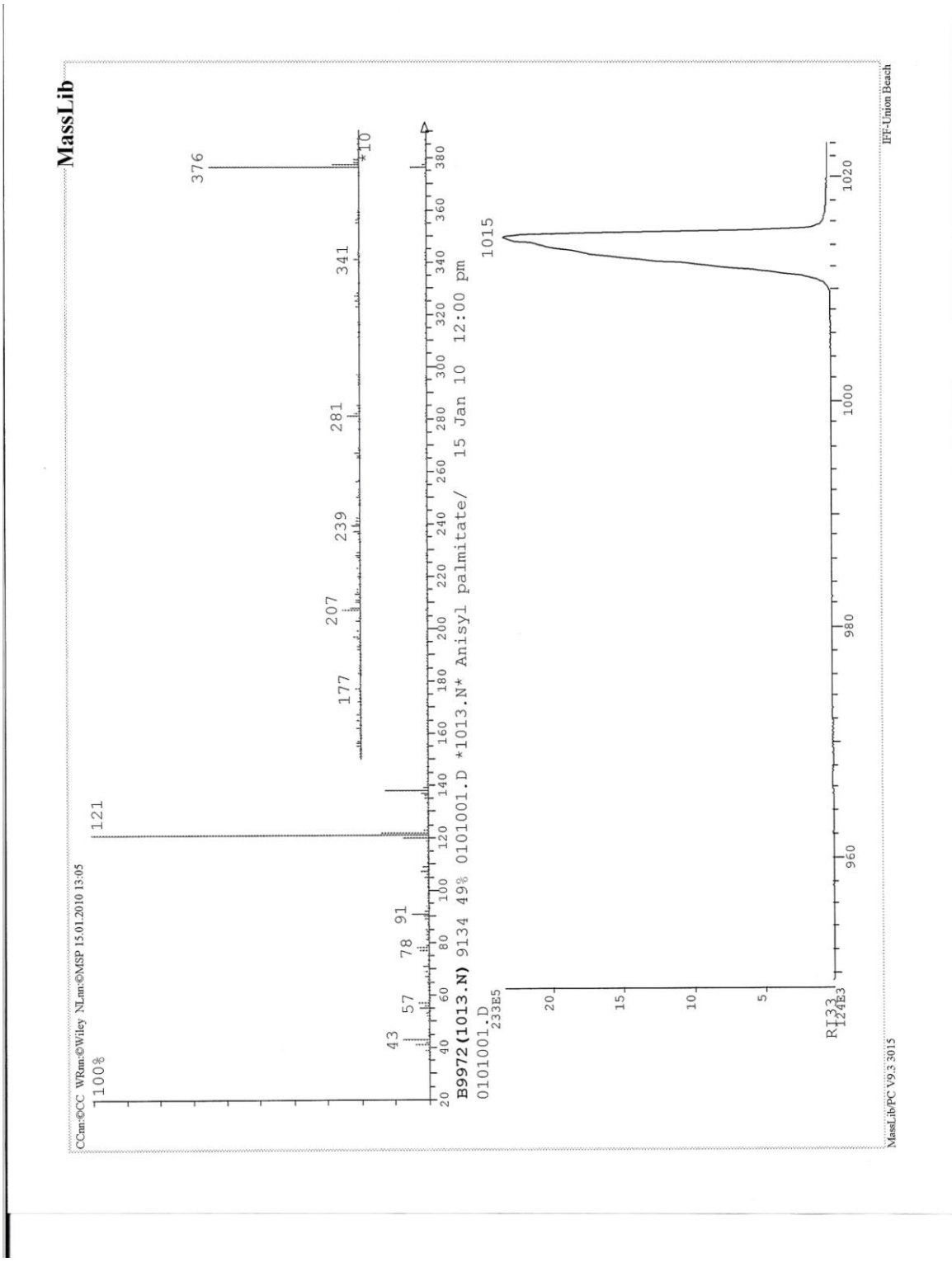
86.513	21.848	919	0.970
86.810	21.928	11	0.011
88.948	22.520	7	0.008
89.993	22.811	8	0.008
90.182	22.864	129	0.136
90.409	22.927	5	0.005
91.635	23.277	13	0.013
94.693	24.158	58050	61.235
96.050	24.548	4	0.005
97.299	24.908	26	0.027
98.341	25.208	13	0.014
100.612	25.862	79	0.083
109.226	28.342	10	0.011
138.344	34.241	396	0.418

NUMBER OF PEAKS FOR SIGNAL 1 = 56
TOTAL AREA FOR SIGNAL 1 = 94798.1647

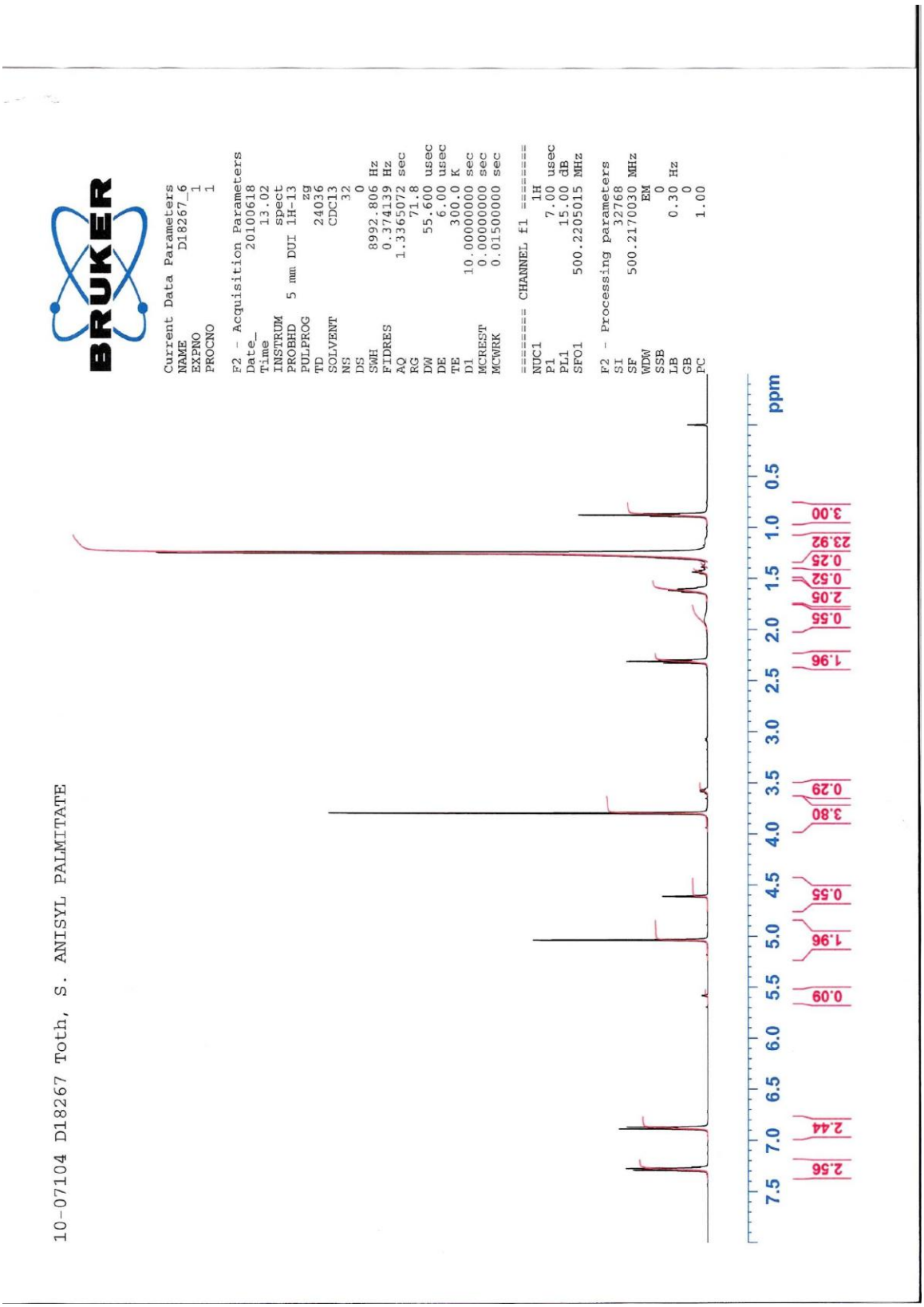
8.11 GC-FID Chromatogram for Anisyl Palmitate



8.12 GC-MSD Chromatogram for Anisyl Palmitate

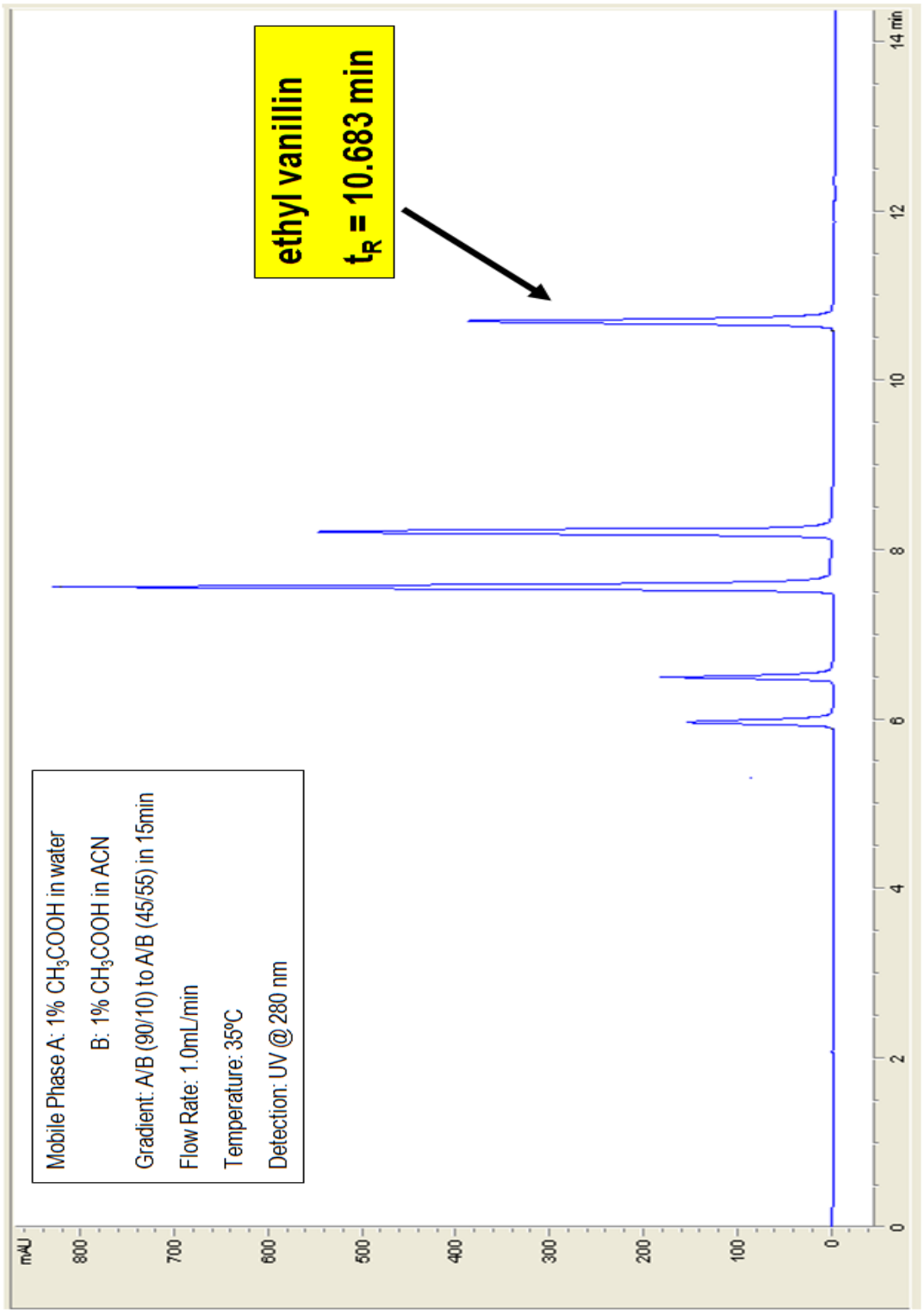


8.13 NMR spectra for Anisyl Palmitate



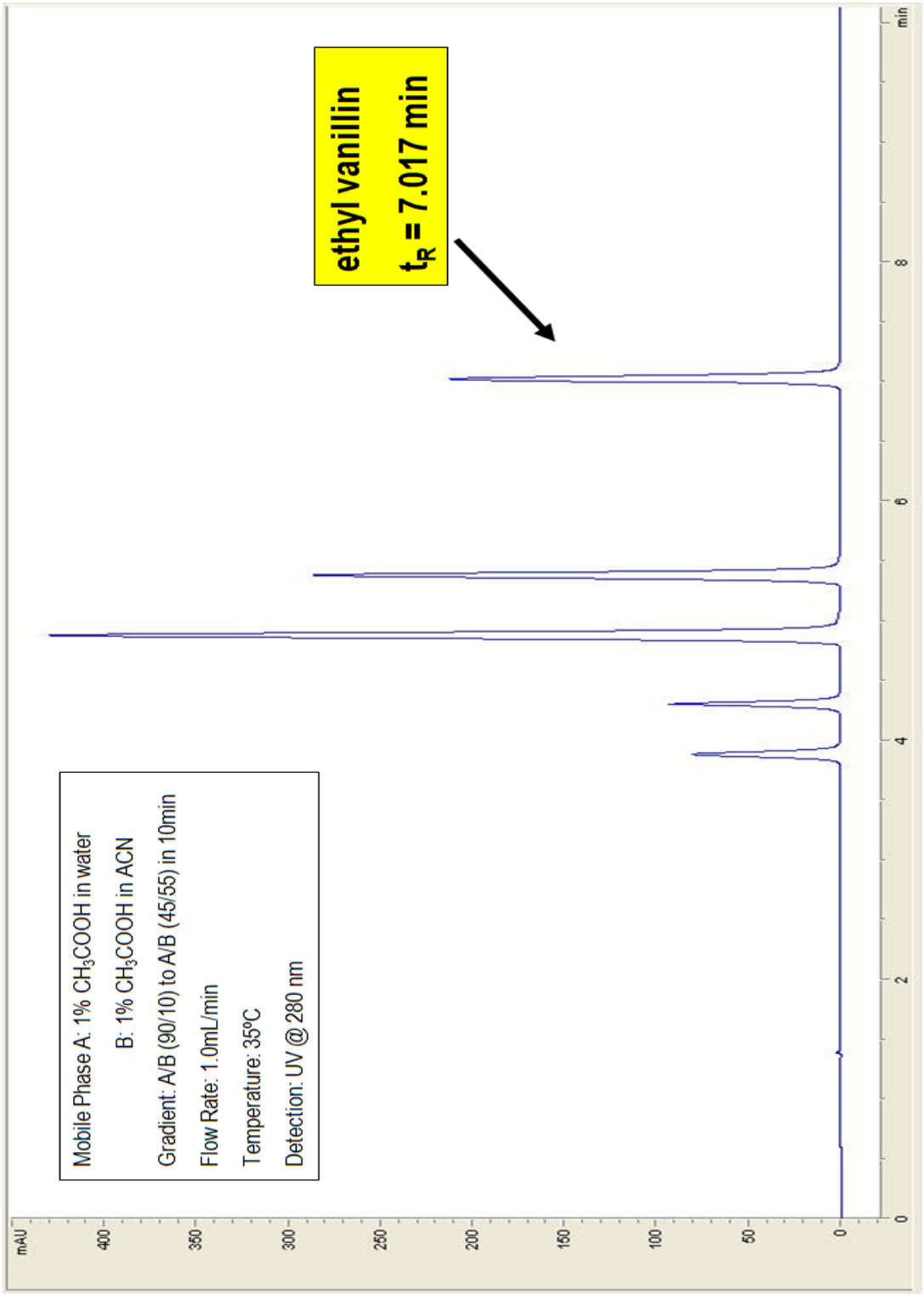
8.14 HPLC chromatogram for Phenomonex Luna C18(2)

150mm x 4.6mm x 3um



8.15 HPLC Chromatogram for Phenomonex Luna C18(2)

100mm x 4.6mm x 3um



8.16 HPLC Chromatogram for Agilent Zorbax Eclipse Plus 100mm x 4.6mm x 1.8um