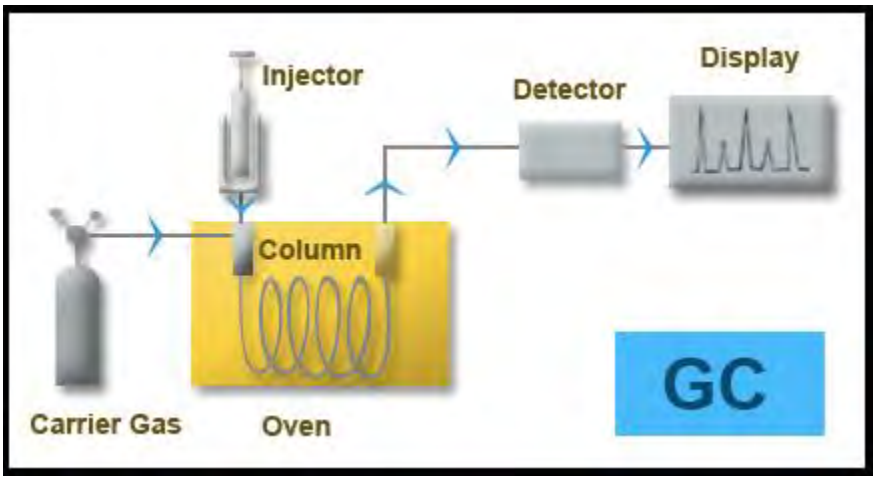
UNDERSTANDING THE PROCESS AND INTERPRETATION OF GC/MS TESTING

By: Sandy Burke

I often see Essential oil companies back their purity claims with an attached GC/MS. GC/MS what is it? Why is it so popular? How does it work? What exactly is this data telling me? I cannot help but wonder why many people insist on buying oils backed by a GC/MS printout believing this sheet of paper guarantees potency and purity even though they do not understand how to interpret the data it contains. In order to understand the whole, I feel that understanding each subsystem is critical. My goal in this analysis is to take away a bit of the mystery surrounding GC, MS, and the "ever so popular" GC/MS. This will give some insight into the amazing world of qualitative and quantitative analysis of essential oil chemistry!

GC is an abbreviation of Gas Chromatography. Gas Chromatography is a versatile instrumentation technique that can be used in most industry areas. The most common areas of use are Pharmaceuticals, petroleum, environmental, chemical manufacturing, food sciences, clinical and forensics. There are multiple components of a GC. The GC contains a carrier gas, injection port, column, oven, a carrier gas, a detector and although not part of the GC, a recorder is necessary for collecting the data. Current modelled Gas Chromatographs also have an electronic interface that is often connected to both the GC as well as the computer system set up to receive the data. This allows the analyst to program the GC remotely and make adjustment to the system parameters as needed. In addition more often than not, moisture traps and regulators are necessary for optimal function of the GC. Many of the components of the GC are interchangeable and can be changed out based on the type of analysis being performed. I have attached a schematic of a basic GC. Please refer to this as each component is being addressed.



schematic provided courtesy of http://lab-training.com

The first major component of the GC is the carrier gas. Much like the gas in your car, it is not part of the car so to speak however your car will not run without it. This can be said for the carrier gas in a GC. The function of the carrier gas is to carry the sample through the system. The Carrier gas is in constant flow and the gas chosen is solely dependent of the analysis being performed and the type of detector being used. The most common carrier gas used for GC is helium. Other gasses used are

hydrogen, nitrogen, air or a mixture of argon and methane. In addition to the type of Gas chosen, the purity of the gas chosen should also be taken into consideration. The higher the purity of the gas the more expensive the gas will be. Choosing the purity of the gas is also dependent on the sensitivity and selectivity of the detector and column chosen.

The next major component of the GC is the injector port. The "injector port" or "inlet," has the very simple purpose of introducing the sample into the carrier gas stream. Components of the inlet include the septa and the glass liner. A septa or septum (both names are appropriate) are small usually silicon pieces specially designed to keep the sample from leaking out after injection. The septa is also used to keep atmospheric air from leaking in to the column which can cause column degradation. There are two types of injector ports: packed and split. A Packed inlet allows the entire sample to be introduced to the column whereas split only allows a portion of the sample to be introduced to the column.

The sample injection can be done either manually or via an automated injecting device. This is often referred to in the industry as an "auto sampler." Approximately 1-3 μL of sample is drawn up into a very precise syringe and then injected through the septa into the heated inlet. The inlet has a glass liner that when the sample is injected into the inlet, instantly vaporizes the sample. The vaporized sample is then mixed with the carrier gas and carried through the column.

The third major component of the GC is the column. Columns for GC use are either packed or capillary. Many components go into column selection such as boiling points of the compounds of interest, polarity of the compounds, compound concentrations, column capacities, and detector used. The main purpose of the column is to separate the components in the analyte. Sample separation occurs as the carrier gas carries the sample through the column based on two factors: sample volatility and analyte polarity. As the compounds elute off the column, they elute off at varying times again based on sample volatility and analyte polarity. This is significant due to the fact that the detector will receive each compound as it elutes off the column thus each compound will be detected and recorded based on the time it elutes off. This is what really makes GC special. GC is highly efficient at separating volatile and semi-volatile compounds with great resolution. GC's major down fall is that it cannot identify the compounds without a known standard to compare the data to.

A brief simplistic summary of GC would be three students go the fair and decide to race each other down the big side. Three students can slide down this huge slide congruently so a race should be no problem. They all sit next to each other and start sliding together. However, one of the students is wearing shorts and unfortunately it's hot out so his skin starts to stick to the slide which slows him down. He gets to the end of the slide last. A second student is wearing cotton pants and slide down the slide at a faster rate but still only comes in second. Last, the third student is wearing slick spandex pants and seems to fly down the slide getting to the bottom very quickly. The speed in which each student travels is based on the attraction their clothing had to the slide even though they started at the same time the time.

Last component of the GC is the detector. The main job of the detector is to detect components in the carrier gas. There are many types of detectors available for use in conjunction with GC. Just a few are: Thermal Conductivity detector, Flame ionization detector, Electron capture Detector and Electrolytic Conductivity detector. For the interest of this analysis we will be highlighting and discussing in detail what is referred to as a Mass-selective Detector. This combination is more commonly known in the industry as Mass Spec. Used together commonly known as GC/MS.

Mass Spectrometry, often abbreviated MS, is used to determine mass-to-charge ratio (abbreviated as m/z or sometime m/Q.) For the sake of simplicity. I will be referring to mass-to-charge as m/z as m/z is the standard when referring to MS of charged particles based on their behavior in magnetic and electric fields. This process is considered a micro-analytical technique and requires some energetic process for converting a significant number of molecules of the analyte to a positively charged form so that the m/z ratio of the charged form of the analyte may be determined. The most common areas of use are Pharmaceuticals, petroleum, biomedical, environmental, chemical manufacturing and forensics. There are multiple components of a MS. The MS contains an ion source, a mass analyzer, and a detector, in addition, a recorder is needed for collecting the data. Once again, current model MS often have an electronic interface that is often connected to both the MS as well as the computer system. I have attached a schematic of a basic MS. Please refer to this as each component is being addressed.

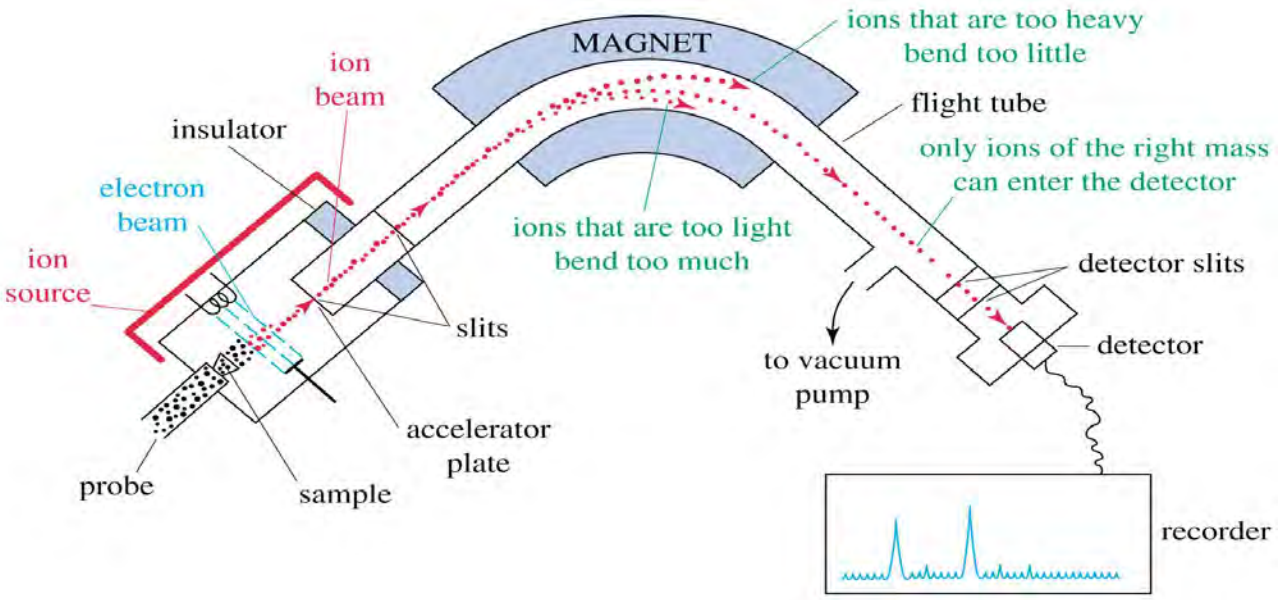


Diagram courtesy of:
http://wps.prenhall.com/wps/media/objects/340/348272/Instructor_Resources/Chapter_12/Text_Images/FG12_15.JPG

MS sample inlet is much like the inlet of a GC. The inlet port is heated such that the analyte is transformed into a vapor upon injection. Once the sample is received by the MS, ionization occurs. Ionization can occur via many ways. A few of which are: Electron Ionization, Chemical Ionization, Cold electron ionization or Mechanism of ionization. For the scope of this paper, we will focus on electron ionization as it is by far the most common and generally considered the standard form of ionization for MS. The ionization occurs in the ion source. It is at this location in the instrument that the sample is bombarded by electrons resonating from a heated filament. The electrons resonating from this filament create a well-defined beam, as depicted in the diagram as “electron beam” (The energy of the electron beam is typically 70 electronvolts). As the sample passes through the electron beam, a process referred to as electron impact or electron ionization occurs. In short, the ion beam causes large

fluctuations in the electric field around the neutral molecules in the analyte and induces ionization and fragmentation of the molecules.

This is where the next component of the MS comes into play. The accelerator plate is sometimes referred to as the “repeller plate” as it repels cations however attracts electrons. The ions and fragments then travel through a pair of slits in the accelerator plates located at the end of the ion source which in addition to accelerating the ions and fragments “herds” the ions and fragments into a beam that is accelerated towards a perpendicular magnet.

The perpendicular magnet field has a magnetic flux density (or gauss) ranging from 500-8000Gs. Ions of different mass then can be focused progressively on to the detector at the end of the MS. This process separates the ions and fragments based on their atomic mass. It is important to note that the internal formations and manipulations of the analyte into ions and fragments must all occur in a vacuum as ions are very reactive and short lived.

A brief simplistic summary of MS would be a simple game of dodge ball where everyone throws the ball at the same speed with the same force; however, the balls differ by size. The bigger balls would bounce off in one direction while each subsequent smaller ball would bounce in another. This would yield a standard result based on the size and the energy of the ball. The main advantage with MS is MS can provide detailed structural information on most compounds at such a high degree that they can be exactly identified. The down fall to MS is it cannot readily separate the compounds.

The GC/MS combination. Now that we understand GC and MS, let’s combine the two and see why the combination has so much synergy. The first thing that needs addressing is how the two instruments come together. The place where the GC and MS connect is referred to as the interface. I have attached a diagram (see below) to show how GC/MS is used in tandem. This can be done in many ways. One of which employs what’s called a jet separator, while in another instance the end of the GC column directly enters the ion source of the mass spectrometer.

In earlier paragraphs I pointed out the main advantage to each of these instruments. GC is highly efficient at separating volatile and semi-volatile compounds with great resolution and the main advantage with MS is MS can provide detailed structural information on most compounds at a high degree. So high in fact they can be exactly identified. This does not, however, explain why GC/MS is such a great combination.

Let’s now look at the major down falls of each instrument. GC major down fall is that it cannot identify the compounds without a known standard to compare the data to. The down fall to MS is it cannot readily separate the compounds. Do you see it now? GC/MS is a fantastic marriage of instrumentation as each strong point compensated for each subsequent instruments downfall. Together GC/MS can successfully separate volatile and semi-volatile compounds with great resolution and provide detailed structural information on most compounds at such a high degree that they can be exactly identified.

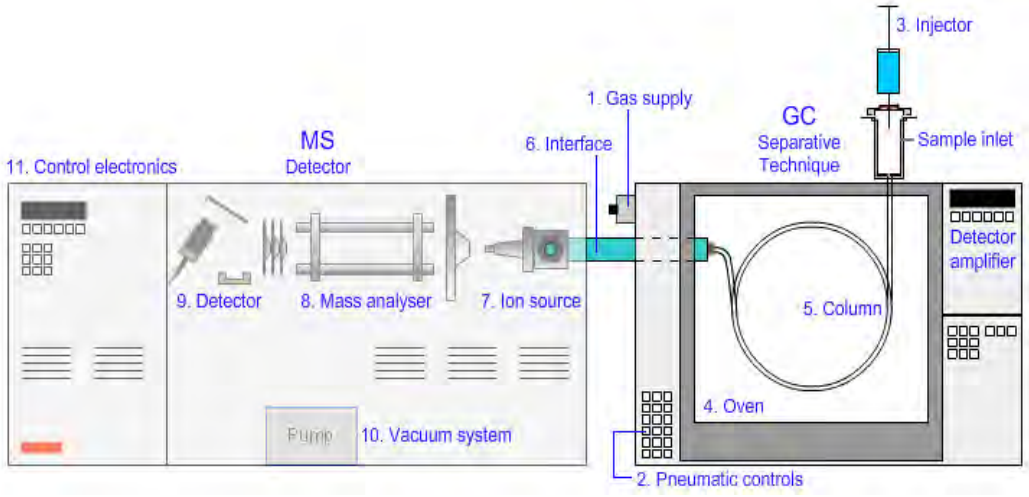


Diagram courtesy of: www.chromacademy.com

Now that we have connected the instrumentation, understand the concepts and its capability's lets discuss the data we receive and how to interpret it. Below I have attached a mass spectrum from a GC/MS run. As you can see this is a GC/MS of Frankincense by a company called Essential Oil University. It is not specified on the mass spectrum what type of Frankincense (Genus species not stated, more about this shortly) is being tested. The top left hand corner of this GC/MS report gives the name of the analyst and Date it was analyzed. It also gives the injection volume. From the data file name I can see that it is listed as Frankincense (again no species listed). To the far right is the TIC Peak report (TIC means total ion mass spectrum and is the detector used for this analysis). The peak report has "R. time" this is the retention time. Retention time is the time between the instant of injection and the detection of the component by the detector. Next, is the name. This represents the name of the constituent found during analysis and just to the right of the name is the Area%. The area % represents the percentage of that constituent found within the sample analyzed. Below the mass spectrum, I have also attached an excerpt from the book "Essential Oil Safety" by Robert Tisserand and Rodney Young. The excerpt has a list of the known constituents in Frankincense (Specifically *Boswellia frereana*). As you can tell you can match the constituents in the mass spectrum with the known constituents in the book. After comparing the Mass Spectrum below it became very clear that the sample analyzed could only be one of two *Boswellia* species: *Boswellia frereana* or *Boswellia sacra*. This is deduced by the fact that the α-Pinene area% is 43.06 (%) per analysis results below. As for all other species of *Boswellia (rivae, neglecta, serrata and papyrifere*) 43.06% would be out of spec.

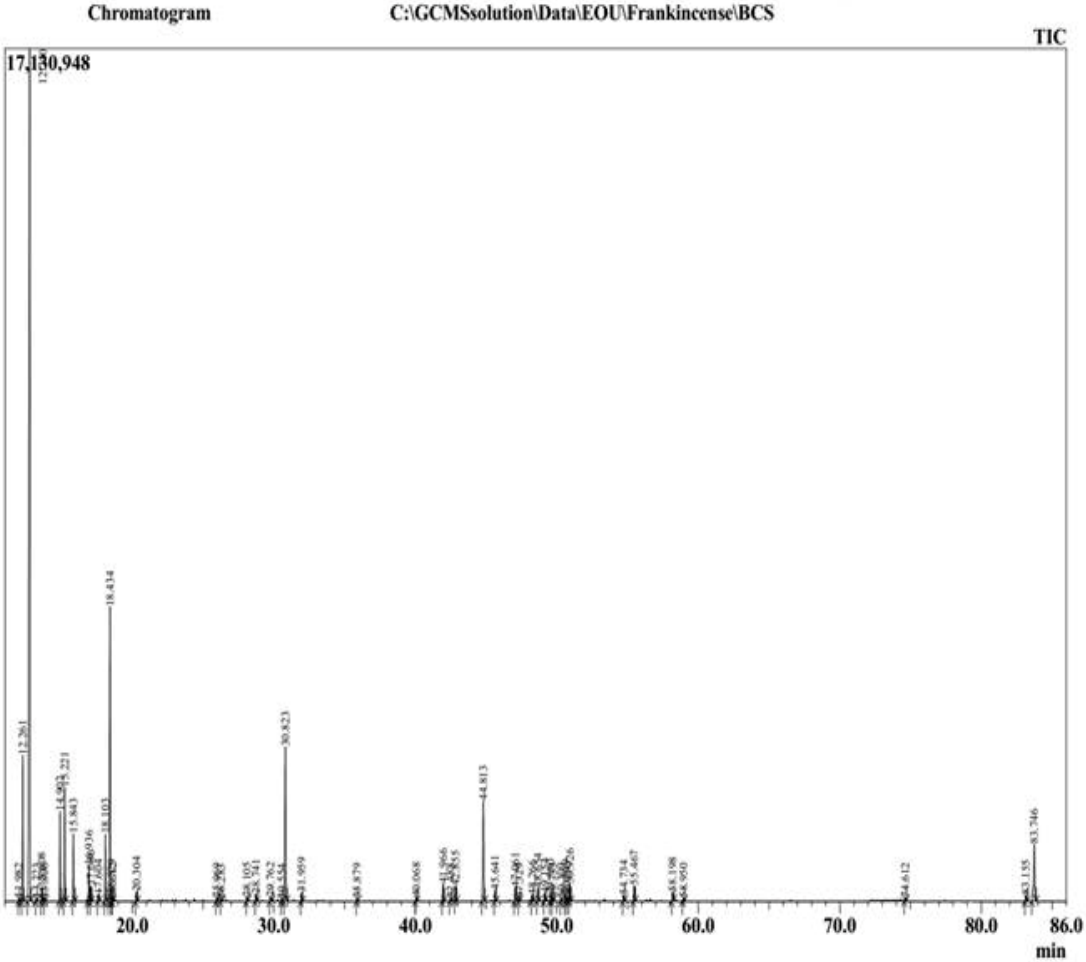
Now, on to the actual mass spectrum. As I mentioned before the retention time is the time between the instant of injection and the detection of the component by the detector. As you can see below each peak has a peak height (how tall the bar rises) and a number next to it (retention time). Those numbers, if you look closely, match the "R. time" value for the constituents on the right. As for the bar height, that is directly related to the % of that constituent found in the analyte. If you recall in a previous section I wrote about the mass-to-charge ratio (m/z). If you look at the mass spectrum across the x-axis you will see a span of numbers. These numbers represent the m/z.
Each bar represents a molecule having that specific mass-to-charge ratio. The length or height of the bar represents the relative abundance of that ion in the analyte. One thing I would like to point out is the mass spectrum is referenced against a mass spectral library. "The library" is an encyclopedic database of "fingerprints" used to identify chemical compounds with a technique called mass

spectrometry “this is a direct quote from the NIST website. Here is the link if you would like more information regarding mass spec library. http://www.nist.gov/srd/library_122205.cfm.

Sample Information

Analyzed by	**: Dr. Robert S. Pappas**
Analyzed	**: 8/29/2014 1:35:44 PM**
Sample Type	**: Essential Oil**
Sample Name	**:**
Sample ID	**:**
Injection Volume	**: 0.10**
Data File	**: C:\GCMSsolution\Data\EOU\Frankincense**

Chromatogram **C:\GCMSsolution\Data\EOU\Frankincense\BCS**



Comments:

Typical Ranges of Main Components:

Peak Report TIC

R.Time	Name	Area%
11.982	Pinene isomer	0.08
12.261	Thujene <alpha->	4.87
12.750	Pinene <alpha>	43.06
13.223	Thujadiene	0.09
13.608	Camphene	0.45
13.806	Thuja-2,4(10)diene	0.08
14.902	Sabinene	3.08
15.221	Pinene <beta->	4.03
15.843	Myrcene	2.36
16.936	Phellandrene <alpha->	1.32
17.080	3-Carene	0.55
17.604	Terpinene <alpha->	0.23
18.103	Cymene <para->	2.50
18.434	Limonene	12.13
18.529	Phellandrene <beta>	0.22
18.612	1,8-cineole	0.12
20.304	Terpinene <gamma>	0.35
25.969	Pinocarveol <trans-(-)>	0.13
26.283	Verbenol <trans>	0.08
28.105	p-Mentha-1,5-dien-8-ol	0.16
28.741	Terpinen-4-ol	0.28
29.762	Terpineol <alpha>	0.24
30.554	Verbenone	0.09
30.823	Octyl acetate	6.82
31.959	Decyl methyl ether	0.37
35.879	Bornyl acetate	0.11
40.068	Cubebene<alpha>	0.17
41.966	Copaene <alpha>	0.78
42.488	Bourbonene <alpha>	0.10
42.855	Elemene <beta>	0.64
44.813	Caryophyllene <trans>	4.84
45.641	Bergamotene <trans-alpha>	0.48
47.061	Humulene <alpha>	0.63
47.329	Alloaromandendrene	0.11
48.266	Cadina-1(6),4-diene <trans>	0.26
48.654	Germacrene D	0.59
49.154	Selinene <beta->	0.32
49.580	Selinene <alpha>	0.33
49.733	Muurolene <alpha>	0.12
50.309	Bisabolene <beta->	0.12
50.619	Amorphene <delta>	0.31
50.750	Cadinene <gamma>	0.15
50.926	Cadinene <delta>	0.77
54.734	Caryophyllene oxide	0.27
55.467	Viridiflorol	0.73
58.198	Cadinol <epi-alpha>	0.39
58.950	Cadinol <alpha>	0.14
74.612	Cembrene A	0.13
83.155	Cembrol<iso>	0.29
83.746	Incensole	3.53
		100.00

Our safety advice

Because of its high α-pinene content we recommend that oxidation of fragonia oil is avoided by storage in a dark, airtight container in a refrigerator. The addition of an antioxidant to preparations containing it is recommended.

Organ-specific effects

Adverse skin reactions: No information found for fragonia oil. Autoxidation products of α-pinene can cause skin sensitization; 1,8-cineole presents only a low risk of both skin irritation and sensitization, and it has antioxidant properties (see 1,8-Cineole profile, Chapter 14).
Reproductive toxicity: The low reproductive toxicity of 1,8-cineole, α-pinene, linalool and (+)-limonene (see Constituent profiles, Chapter 14) suggests that fragonia oil is not hazardous in pregnancy.

Systemic effects

Acute toxicity: No information found for fragonia oil. None of its key constituents is significantly toxic (see Constituent profiles, Chapter 14).
Carcinogenic/anticarcinogenic potential: No information found for fragonia oil, but it contains no known carcinogens. (+)-Limonene displays anticarcinogenic activity (see (+)-Limonene profile, Chapter 14).

Comments

The name 'fragonia' has been trademarked by the sole producer of this oil in Australia.

Frankincense

Synonym: Olibanum
Botanical names: *Boswellia frereana* Birdwood (synonym: *Boswellia hildebrandtii* Engl.) (African elemi, elemi frankincense); *Boswellia papyrifera* (Del.) Hochst (synonyms: *Amyris papyrifera*, *Boswellia chariensis*, *Boswellia occidentalis*, *Boswellia odorata*, *Ploesslea floribunda*) Sudanese frankincense; *Boswellia sacra* Flueck. (synonyms: *Boswellia carteri* Birdwood, *Boswellia bhau-dajiana* Birdwood, *Boswellia undulato-crenata*) Saudi frankincense; *Boswellia serrata* Roxb. (synonyms: *Boswellia glabra* Roxb., *Boswellia thurifera* Roxb., *Chloroxylon dupada*) Indian frankicnese; *Boswellia neglecta* S. Moore ('Borena' type frankincense); *Boswellia rivae* Engl. ('Ogaden' type frankincense)
Family: Burseraceae

Essential oil

Source: Gum resin
Key constituents:

Boswellia frereana

α-Pinene	41.7–80.0%
Sabinene	0.5–21.0%
α-Thujene	0–19.3%
(+)-Limonene	0–17.0%
Viridiflorol	0–15.2%
p-Cymene	0.7–11.7%
β-Pinene	0–6.9%
Verbenone	0–6.5%
β-Myrcene	0–6.0%
α-Phellandrene	0–5.9%
Bornyl acetate	0–5.6%
Carvone	0–4.4%
δ-3-Carene	0–3.4%
Linalool	0–3.0%
1,8-Cineole	0–2.9%
γ-Terpinene	0–2.5%
Camphene	0–2.1%
Thujol	0–1.8%
α-Pinocarveol	0–1.7%
Campholenic aldehyde	0–1.5%
Octyl acetate	0–1.5%
α-Terpinene	0–1.5%
β-Elemene	0–1.3%
β-Caryophyllene	0–1.2%
α-Thujone	0–1.2%
(Z)-β-Ocimene	0–1.0%
β-Phellandrene	0–1.0%
β-Selinene	0–1.0%

(Tucker 1986; Lawrence 1995g p. 20–23; Hall 2000)

Boswellia papyrifera

Octyl acetate	50.0–60.0%
1-Octanol	3.5–12.7%
Terpinen-4-ol	0–8.0%
(+)-Limonene	1.7–5.0%
α-Pinene	1.0–4.6%
Incensyl acetate	3.0–4.1%
Cadinol	0–3.0%
Incensol	2.1–2.7%
Thymol	0–2.6%
Linalool	0.2–2.5%
Cembrene A	1.4–2.3%
Isocembrene	0–1.8%
1,8-Cineole	0–1.6%
(*E*)-β-Ocimene	1.3–5%
α-Thujene	0–1.4%
Bornyl acetate	1.0–1%
Camphene	0–1.1%

(Tucker 1986; Lawrence 1995g p. 20–23; Hall 2000)

Boswellia sacra (α-pinene CT)

α-Pinene	10.3–51.3%
α-Phellandrene	0–41.8%
(+)-Limonene	6.0–21.9%
β-Myrcene	0–20.7%

GC/MS is the standard for essential oil analysis. It is used for both qualitative identification as well as quantitative measurement for volatile and semi-volatile organic compounds. I want to point out that this is not an exhaustive paper on GC, MS or GC/MS. This is merely a vague overview. My intent is that the reader will be able to understand what they are looking at when they see or receive a mass spectrum of an essential oil. I love the fact that essential oils have gained the so much attention. I also love the fact that people are yearning to educate themselves about these fascinating oils. I hope this can heighten the interest of essential oil enthusiasts to review the mass spectrum they receive and I hope this analysis gives them the power to be discerning about the products they choose to purchase.

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“Essential Oil Safety” by Robert Tisserand and Rodney Young.