Gas Chromatography for Separation of Natural Products from Different Plant Materials[1]

Dr. K. Venkata Krishna
Assistant Professor of Chemistry
Government Degree College for Women (A), Nalgonda, Telangana, India

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ABSTRACT

Gas chromatography separates compounds that evaporate easily from plant materials. These compounds form the main part of essential oils and include monoterpenes, sesquiterpenes, and their oxygenated forms. This paper explains how the technique works for different plant parts. It describes sample preparation methods such as hydrodistillation and solvent extraction. It lists common column types and temperature programs. Examples from mint, basil, eucalyptus, citrus, and ginger show how the method identifies major compounds. The approach helps check oil quality, track seasonal changes, and link chemical profiles to biological activity. Matching the isolation step and the column to the sample produces clear, useful results.

Keywords: *gas chromatography; GC-MS; essential oils; terpenoids; plant extracts; sample preparation; retention index*

1. Introduction

Plants store many small molecules that give them scent, flavor, and biological activity. Most of these molecules boil at moderate temperatures and travel with steam or evaporate into the air. Chemists call them volatile natural products. They belong mainly to two groups: monoterpenes with ten carbon atoms and sesquiterpenes with fifteen carbon atoms. Oxygen atoms attached to these frameworks create alcohols, ketones, and esters that change both smell and solubility.

Gas chromatography separates these compounds because they form a gas when heated. A thin coated tube, called a capillary column, sits inside an oven. Carrier gas pushes the sample through the tube. Compounds that stick weakly to the coating exit first. Compounds that stick more strongly exit later. A detector records each compound as a peak on a chart. Mass spectrometry identifies the structure of the molecule that produced the peak. Flame ionization measures how much of each compound is present.

The method has become standard for essential-oil work because one run can separate dozens of compounds. Researchers use the profiles to compare plant varieties, check commercial oils for adulteration, and select materials for further biological testing. This paper reviews the practical steps that produce reliable profiles from different plant materials.

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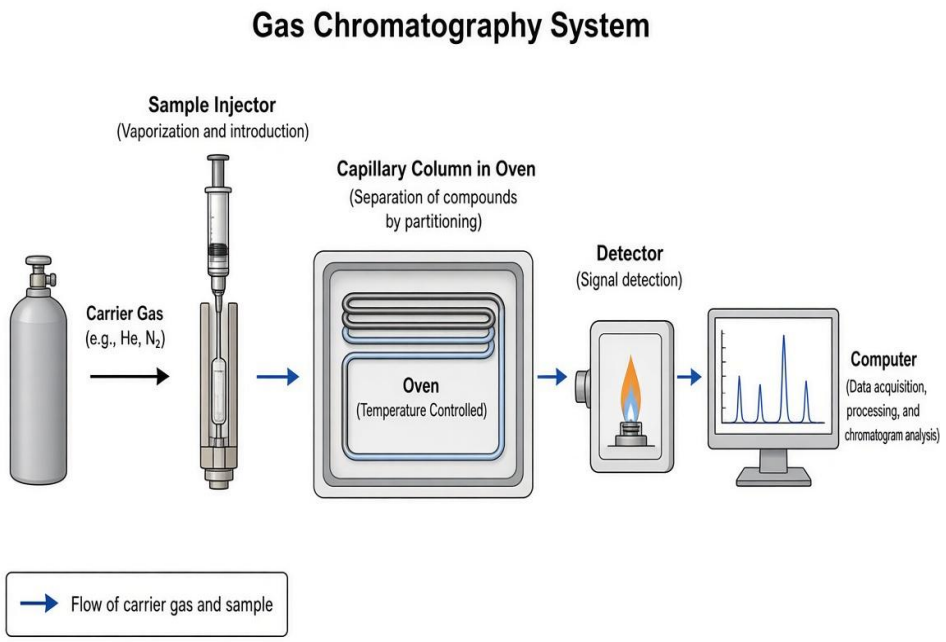


Figure 1. Schematic diagram of a gas chromatography system.

2. Separation Principles and Column Choice

The coating inside the column controls the order of peaks. A non-polar coating made of dimethylpolysiloxane with a small amount of phenyl groups sorts compounds largely by boiling point. Most monoterpene hydrocarbons separate well on these columns. A polar coating made of polyethylene glycol adds interactions based on polarity. Oxygenated compounds such as alcohols and ketones interact more strongly and often separate better on polar columns.

Many laboratories run the same sample on both a non-polar and a polar column. The two sets of retention times help confirm identities when mass spectra look similar. Temperature programming is required for complex mixtures. The oven starts cool so light monoterpenes leave early. The temperature rises slowly so heavier sesquiterpenes still form sharp peaks. A common program holds at 60 °C for a few minutes, then rises 3–5 °C per minute to 240 °C and holds again.

Scientists calculate a retention index for each peak so results can be compared between laboratories. The Kováts index uses the retention times of nearby alkanes:

$$I = 100 \times [n + (\log t'R(x) - \log t'R(n)) / (\log t'R(n+1) - \log t'R(n))]$$

where t′R is the adjusted retention time and n is the carbon number of the alkane that elutes just before the unknown. The index stays nearly constant even when the exact temperature program changes slightly.

3. Sample Preparation Methods

Hydrodistillation remains the most common way to obtain essential oils from leaves and flowers. Steam passes through the plant material and carries volatile compounds into a condenser. The oil separates from water and is collected. Fresh mint leaves and eucalyptus leaves give good yields with this method. Dried material yields less oil but often produces more consistent results because water content is lower.

Solvent extraction works better for roots, seeds, and bark that contain less oil. Hexane or ethyl acetate dissolves the target compounds. The solvent is later removed under reduced pressure. Headspace solid-

phase microextraction needs no solvent and works on small fresh samples. A fiber coated with adsorbent sits above the sample and collects volatiles for a set time. The fiber is then placed directly in the injector.

After isolation, residual water is removed with anhydrous sodium sulfate. The extract is filtered and diluted. An internal standard improves quantitative accuracy. Compounds that do not evaporate easily, such as phenolic acids, require a chemical change called silylation before injection. The reaction replaces the hydrogen of an OH group with a trimethylsilyl group:

R–OH + BSTFA → R–O–Si(CH₃)₃ + by-products

The modified molecule evaporates more readily and travels through the column without excessive tailing.

4. Typical Chromatographic Conditions

Most essential-oil work uses a 30 m column with 0.25 mm inside diameter and 0.25 µm film thickness. The injector temperature is set between 220 and 250 °C. The detector temperature is set between 250 and 280 °C. Helium flows at 1.0–1.5 mL per minute. Split ratios of 50:1 to 100:1 keep the amount of sample small when the oil is concentrated. Dilute extracts may use splitless injection.

A representative temperature program holds at 60 °C for 2–5 minutes, rises at 3–4 °C per minute to 220–240 °C, and holds for 5–10 minutes. Shorter columns (10–15 m) and faster ramps finish a run in under 15 minutes when only the major peaks are needed. Dual detection with both flame ionization and mass spectrometry provides both quantity and identity in related runs.

5. Applications to Different Plant Materials

5.1 Mint (Mentha species)

Mint leaves produce oils rich in menthol and menthone. Indian accessions grown on the northern plains often contain 30–45 % menthol and 15–30 % menthone. The two compounds separate cleanly on a non-polar column. Retention indices and mass spectra confirm their identities. High-altitude collections sometimes show higher menthofuran or pulegone. These differences matter for commercial quality because menthofuran and pulegone levels affect the oil’s acceptability.

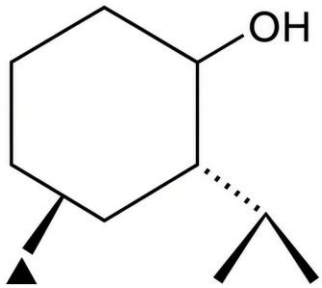


Figure 2. Structure of menthol, a major compound in mint oil.

5.2 Basil and tulsi (Ocimum species)

Ocimum species display several distinct chemotypes. Some basil oils contain more than 50 % methyl chavicol. Tulsi (Ocimum sanctum) usually contains high levels of eugenol. Camphor dominates in certain other species. Polar columns help separate these oxygenated compounds when they overlap on non-polar phases. Multivariate analysis of peak areas can group populations by chemotype and support breeding programs.

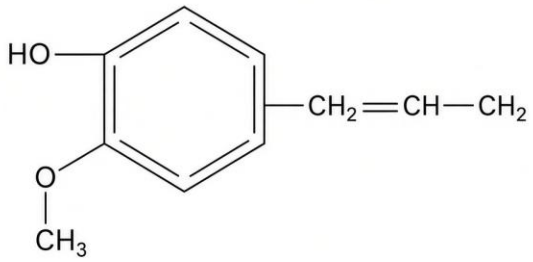


Figure 3. Structure of eugenol, common in tulsi essential oil.

5.3 Eucalyptus leaves

Eucalyptus globulus leaf oil often contains 70–85 % 1,8-cineole. Other species contain more p-cymene or pinene. Summer harvests of certain clones give higher cineole and stronger antifungal activity against plant pathogens. GC-MS measures these seasonal shifts directly and links chemical composition to biological effect.

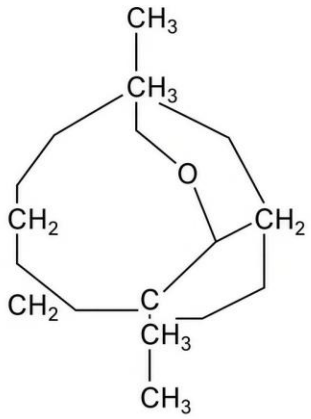


Figure 4. Structure of 1,8-cineole (eucalyptol).

5.4 Citrus peel and ginger rhizome

Citrus peel oils are dominated by limonene, often above 60–70 %. Other monoterpenes such as myrcene and linalool appear in smaller amounts. Ginger rhizome oils contain high levels of sesquiterpenes such as zingiberene, β-sesquiphellandrene, and α-curcumene. These heavier compounds require the higher end of the temperature program for complete elution. The profiles help identify geographic origin and detect adulteration with cheaper oils.

Table 1. Major compounds and typical ranges in selected essential oils

Plant source	Main compounds	Typical range (%)
Mentha piperita leaf	Menthol, menthone	30–45, 15–30
Ocimum basilicum aerial	Methyl chavicol	>50 in some types
Eucalyptus globulus leaf	1,8-Cineole	70–85
Ocimum sanctum leaf	Eugenol	40–75
Citrus peel	Limonene	60–90
Zingiber officinale rhizome	Zingiberene	20–40

Ranges come from published analyses of Indian and regional collections. Exact values change with plant variety, season, and isolation method.

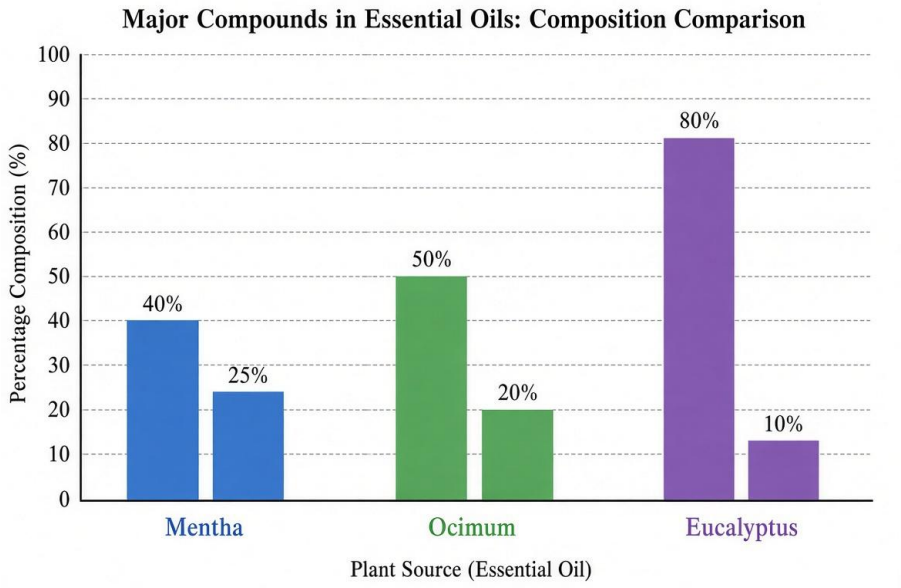


Figure 5. Comparison of major compound levels in three essential oils (illustrative values).

6. Linking Composition to Biological Activity

Many studies run biological tests on the same oils that are profiled by GC-MS. Antioxidant activity is often measured with DPPH or ABTS assays. Antimicrobial activity is tested against bacteria and fungi by disk diffusion or minimum inhibitory concentration methods. Oils high in menthol, eugenol, or 1,8-cineole frequently show stronger activity. Seasonal or chemotypic differences that appear in the chromatogram often match differences in biological effect. This connection helps select promising plant materials for further work.

7. Limitations and Recent Advances

The method cannot analyze compounds that do not evaporate without extra chemical steps. Some fragile molecules break down in the hot injector. Peak-area percentages from the flame ionization detector are relative rather than absolute. Absolute amounts require calibration with pure standards. Comprehensive two-dimensional gas chromatography increases peak capacity and resolves more minor components. Faster temperature ramps and shorter columns reduce analysis time for routine quality control. Ionic-liquid stationary phases offer different selectivity for critical pairs that co-elute on conventional columns.

8. Conclusion

Gas chromatography separates and identifies the volatile natural products found in many plant materials. Careful choice of isolation method and column type produces reliable profiles. These profiles support quality control, track seasonal and geographic variation, and guide the search for useful compounds. Further improvements in speed and peak capacity will keep the method useful for complex plant extracts.

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