Unique Separation of Mint Essential Oils by Gas Chromatography Mass Spectrometry Using Two Different Capillary Phases: Bonded Polyethylene Glycol and a Novel Ionic Liquid Phase

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Extracts of peppermint and spearmint oils were analysed by gas chromatography mass spectrometry using two different capillary phases: bonded polyethylene glycol and an ionic liquid phase. The bonded polyethylene glycol is a polar phase commonly used for the analysis of flavour and fragrance compounds and especially for mint oils. The ionic liquid phase is 1,12-di(tripropylphosphonium)dodecane bis(trifluoromethylsulfonyl) imide liquid phase (SLB®-IL60i). The ionic liquid phase is slightly more polar which may result in unique elution patterns, is more inert and can go to a higher maximum temperature compared to the polyethylene glycol phase. The gas chromatography mass spectrometry comparison will illustrate differences in separation and retention time.

Introduction

An overview of two similar polar phases will be examined for analysing essential oils. This overview provides a fingerprint elution order for peppermint and spearmint oils using the polyethylene glycol and ionic liquid phases and it also highlights the uniqueness in elution order when using an ionic liquid capillary column. The ionic liquid phase is a new dicationic phosphonium cation liquid stationary phase which has selectivity like a polyethylene glycol (PEG) or wax phase. A PEG type phase is traditionally used for essential oil analysis [1]. However, the PEG phase does not always perform a few key separations. Over the years, extensive evaluations of columns manufactured with ionic liquid stationary phases has occurred [1,2,3]. Their main strength was discovered to be unique selectivity, higher temperatures and minimum column bleed compared to the PEG column. These columns have the ability to perform many of the same applications as columns made with polyethylene glycol stationary phases but with slight elution order changes. Many times, this results in increased resolution and/or shorter run time.

Essential oil analyses are challenging to chromatographers because of the complexity of the various samples. The quality and authenticity of the oil needs to be evaluated by analytical means to ensure the product's description reflects its true composition and that the capillary column is providing adequate separation. The best way to improve the resolution of essential oil samples is to choose a capillary column with a unique selectivity that is stable and reproducible. Traditionally, columns based on polyethylene glycol chemistries have been the columns of choice for these analyses due to the separation and sufficient analysis time. It is important to demonstrate the compounds resolution because different species of peppermint (Willamette vs. Yakima) or spearmint (Farwest Native vs. Farwest Scotch) oils may contain different percentages or ratios of the key flavour and fragrance compounds.

Peppermint and spearmint oils are aromatic liquids that are extracted from the plant leaves by steam distillation. The distinctive aroma of essential oils is attributed to the unique composition of chemicals in the plant which includes a wide variety of chemical compounds such as: terpenes, ketones, aldehydes, alcohols, acids, esters, and hydrocarbons [4].

Peppermint is cultivated in Oregon (Willamette Valley) and Washington State (Yakima Oil). Northern regions are considered to produce the best quality of oil due to the long daylight hours. Composition of the oil is influenced by many factors: cultivation, production, geographic location, time of planting and harvest, and the distillation process. Under short day conditions mint plants have been observed to produce oil with higher levels of menthofuran and lower levels of methone and menthol than plants in long-day conditions (5). The main components of Peppermint oil are menthol (peak 30) and menthone (peak 31) in Figures 1 and 2. Yakima Oil contains higher levels of methofuran. Methofuran has a tendency to autoxidize and may be added back into the final peppermint to restore the peppermint characteristic. p-Cymene (peak 13, Figures 1 and 2) is also of interest in peppermint oil because it is a measure of oxidation [5].

Willamette Peppermint has a fresh minty, non-herbaceous odour that is cool and refreshing while Yakima peppermint is pungent and herbal in taste and smell. There is a variety of uses for peppermint oil such as adding flavour or fragrance to foods, cosmetics, soaps, toothpastes, mouthwashes, and other products.

The aromatic fragrance of Farwest Native and Farwest Scotch spearmint oil is immediately recognised for its crispness,

and is used to flavour food, popular drinks like iced tea, alcoholic beverages, and candy. Carvone is what gives spearmint its distinctive smell, which is often associated with cleanliness, making it popular for use in mouthwash, shaving creams, soaps, and shampoos.

The ionic liquid phase provides unique selectivity of essential oil compounds (i.e. terpineol-4-ol/E- Caryophyllene/ neo-Menthol) that are different from the traditional PEG columns (Figure 3). Ionic liquid phases are much smaller compared to big, bulky polysiloxane polymers and polyethylene glycol phases, plus there are no active hydroxyl groups. These features lead to greater stability, even in the presence of moisture and/or oxygen. The ionic liquid phase undergoes the same analyte-phase interactions as the polyethylene glycol but at different relative amounts. The ionic liquid phase also undergoes additional interactions that the PEG phase cannot. With PEG columns, possible interactions appear to be dispersive, hydrogen bonding, and acid-base interactions and with the ionic liquid phase possible interactions appear to be dispersive, dipole-dipole, dipole-induced dipole, pi-pi, hydrogen bonding and acid-base interactions. Due to these additional interaction mechanisms, the ionic phase will retain some polar and polarisable analytes relatively longer, and some non-polar analytes relatively less. This results in unique and alternate selectivity compared to traditional PEG columns.

While the main components of Spearmint oil are α-pinene, β-pinene, carvone, eucalyptol, linalool, limonene, myrcene, (E)-caryophyllene and menthol (~0.5% menthol is reported in spearmint compared to the ~40.0% in peppermint oil). Pure spearmint oil contains at least 45-80% of carvone and 10-30% of limonene. Native spearmint oil (Figure 4), for example, contains an amount of trans-sabinenehydrate (peak 24) and no methone (peak 31), while the opposite is true for Scotch spearmint oil (Figure 7). There is a large amount of methone and no trans-sabinenehydrate. The ratio of trans-sabinenehydrate to methone is a useful way to distinguish between the two oils.

Results and Discussion

This work compared the selectivity of the ionic liquid stationary phase with a traditional phase of similar or like selectivity for the analysis of peppermint and spearmint essential oil samples.

Experimental

Mint Oil GC-MS Conditions

Instrument:	Shimadzu GC-MS 2010 QP single quad equipped with a split-splitless inlet
Column:	bonded Polyethylene glycol phase 30 m x 0.25 mm x 0.25 µm Ionic Liquid phase (SLB®-IL60i) 30 m x 0.25 mm x 0.20 µm
Detector:	GC-MS, Scan, Ion Source: 200°C, Interface Temp.: 200°C
Inlet:	250°C
Column Temperature:	40°C (4 min) @ 4 degrees/minute to 260°C (30 minutes)
Flow:	30.0 cm/sec Helium at constant pressure at 40°C
Liner:	Splitless with wool
Split Ratio:	200:1
Sample size:	1µl
Sample:	Mint Oil

100 µl mix into 1ml Methanol or Hexane

Mint Essential Oil Elution

1. alpha Pinene
2. Camphene
3. beta Pinene
4. Sabinene
5. Myrcene
6. Limonene
7. alpha Terpinene
8. (Z)-β-Ocimene
9. γ-Terpinene
10. Terpinolene
11. Eucalyptol
12. (E)-β-Ocimene
13. p-Cymene
14. 3-Octanol
15. 1-Octene-3-ol
16. Butanoic acid 2-methyl-2-methylbutyl ester
17. n-amyl Isovalerate
18. 3-Octanol acetate
19. cis-Sabinenehydrate
20. 2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a- tetramethyl-(2.alpha,4a.beta,8a.beta)
21. Methofuran/Linalool
22. β-Bourbonene
23. (E) p-Menth-2-en-1-ol
24. trans-Sabinenehydrate
25. β-Elemene/Germacrene
26. Terpineol-4-ol
27. neo-Menthol
28. iso-Menthol
29. (E)-Caryophyllene
30. Menthol
31. Menthone
32. neo-Menthyl acetate
33. Menthyl acetate
34. iso-Menthone
35. Cyclohexanol,5-methyl-2-(1-methylethyl)-acetate (1.alpha,2.alpha,5.alpha)
36. Germacrene D
37. Bicyclogermacrene
38. Pulegone
39. Ocimenol
40. Piperitone
41. Virdiflorol
42. cis-beta-Terpineol
43. β-Cubebene
44. trans-β-Farnesene
45. Neodihydrocarvel
46. alpha Terpineol
47. trans Carveol
48. Dihydrocarvone
49. cis Carveol
50. trans Carveyl acetate
51. Carvone
52. Jasmone
53. n-Valeric acid cis-3-hexenyl ester
54. Limonene 1,2 epoxide
55. 3-Octyl acetate
56. Isomenthyl acetate

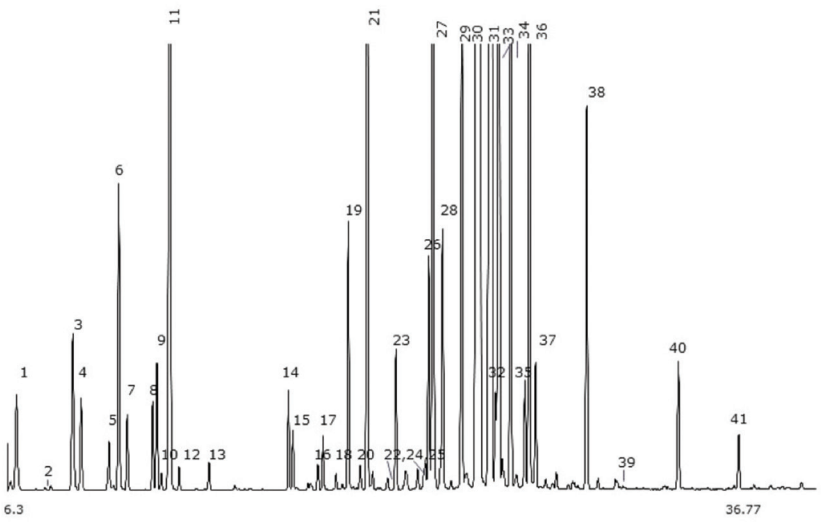

Figure 1: Willamette Peppermint Oil Natural, ionic liquid column.

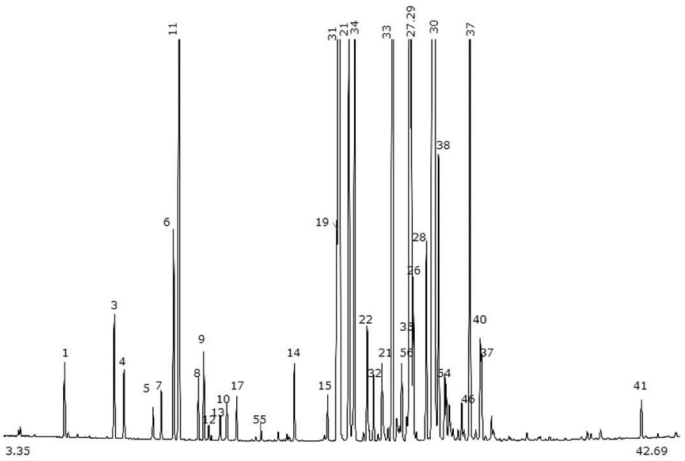

Figure 2: Willamette Peppermint Oil Natural, bonded polyethylene glycol column.

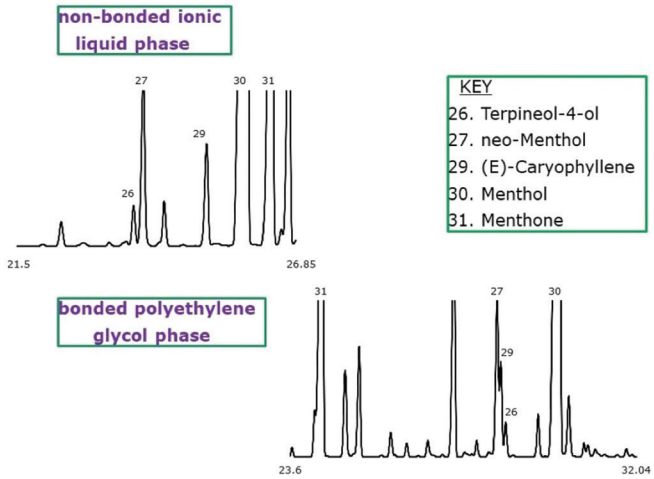

Figure 3: Phase Comparison of Willamette Peppermint Oil Natural Enlargement.

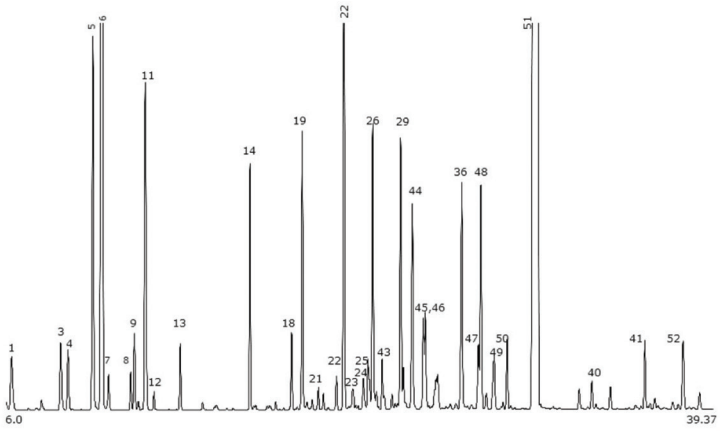

Figure 4: Farwest Native Spearmint Oil Natural, ionic liquid column.

Table 1: Comparison of the main components of Willamette Peppermint Oil using two different polar phases.

Comparison of Capillary Phases	Ionic liquid Phase	Bonded Polyethylene Glycol Phase
Retention Time	~36.77 minutes	~42.69 minutes
Separation of Five Major Components in Peppermint Oil (terpineol-4-ol (pk 26), neomenthol (27), (E)-caryophyollene (29), menthol (30), menthone (31))	Baseline separation of all five components	Baseline separation of two components (menthone and menthol)
Baseline	No baseline rise (maximum temperature 280 °C)	No baseline rise (maximum temperature 280 °C)
Unique Separations	Terpineol-4-ol and menthone Peaks 26 and 31	Terpineol-4-ol and menthone Peaks 31 and 26

Samples of the essential oils were obtained from a commercial source (A.M. Todd, a globally recognised company who ensures quality and integrity in mint oils). A GC-MS 2010 QP Shimadzu single quad equipped with a split-splitless injector was used in the method development highlighting two polar phases with different selectivity i.e. ionic liquid phase and bonded polyethylene glycol phase. The aim of our work was to analyse the essential oils and see if there were any unique separation patterns using the two different polar capillary column phases.

There were significant differences in chemical composition observed between peppermint and spearmint essential oils. Willamette Peppermint Natural essential oil (Figures 1 and 2) included the five major components: terpinene-4-ol (peak 26), neo-menthol (peak 27), (E)-Caryophyllene (peak 29), menthol (peak 30), and menthone (peak 31). Farwest Native Spearmint Natural essential oil sample's (Figures 4 and 5) were abundant in limonene (peak 6), eucalyptol (peak 11), dihydrocarveol (peak 48) and carvone (peak 51).

Table 1 summarises the comparison of main components in Willamette Peppermint Oil using two polar phases. The ionic liquid phase provided good resolving power toward all classes of compounds present in the Willamette Peppermint oil and the ionic liquid phase had baseline separation of all five major components (terpineol-4-ol (peak 26), neo-menthol (peak 27), (E)-caryophyllene (peak 29), menthol (peak 30), and menthone (peak 31)) compared to the resolution of the same compounds using the bonded PEG phase (Figure 3). The bonded polyethylene glycol phase was unable to baseline resolve neo-Menthol/(E)-Caryophyllene/terpineol-4-ol (Figure 3). The polar ionic liquid column has a polarity/selectivity like that of polyethylene glycol columns but different enough to provide a unique elution pattern for terpineol-4-ol and menthone (Figure 3). Another advantage of the ionic liquid phase was the lower bleed after repeated injections of the mint oils when reaching the column maximum temperature limit of 280°C. Farwest Native Spearmint Oil illustrates the increase in bleed after repeated injections on the PEG column (Figure 5). In both the Peppermint and Spearmint oils the ionic liquid phase had the faster retention time without losing resolution. This is illustrated in Figures 1 and 4 compared to Figures 2 and 5.

There are several examples of unique selectivity in the analysis of Farwest Native Spearmint Oil Natural with the ionic liquid phase column. The first example is summarised in Table 2 and illustrated

in Figure 6. Figure 6 demonstrates the distinctive separation of compounds (Z) β-ocimene (peak 8), γ-terpinene (peak 9), eucalyptol (peak 11), and (E) β-ocimene (peak 12). Eucalyptol elutes before (Z) β-ocimene on the PEG column and eucalyptol elutes after (Z) β-ocimene on the ionic liquid column.

Another unique example of selectivity is alpha terpinene. The polyethylene glycol column (Figure 5) has alpha terpinene (peak 7) eluting before limonene (peak 6) and the ionic phase column (Figure 4) has alpha terpinene (peak 7) eluting after limonene (peak 6).

The third example of selectivity is illustrated in Figures 4 and 5 regarding compounds dihydrocarvone (peak 48), cis carveol (peak 49), and trans carveyl acetate (peak 50). Trans carvel acetate elutes between peaks 48 and 49 on the polyethylene glycol phase where trans carvel acetate elutes after peaks 48 and 49 on the ionic phase.

The final example of selectivity is shown in Figures 4 and 5 with virdiforol (peak 41) and jasmone (peak 52). Jasmone elutes before virdiforol on the bonded polyethylene glycol phase and virdiforol elutes before jasmone on the ionic liquid phase.

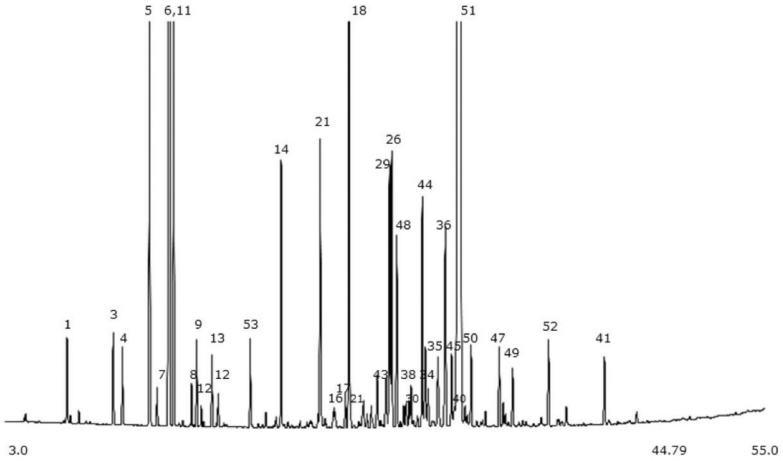

Figure 5: Farwest Native Spearmint Oil Natural, bonded polyethylene glycol column.

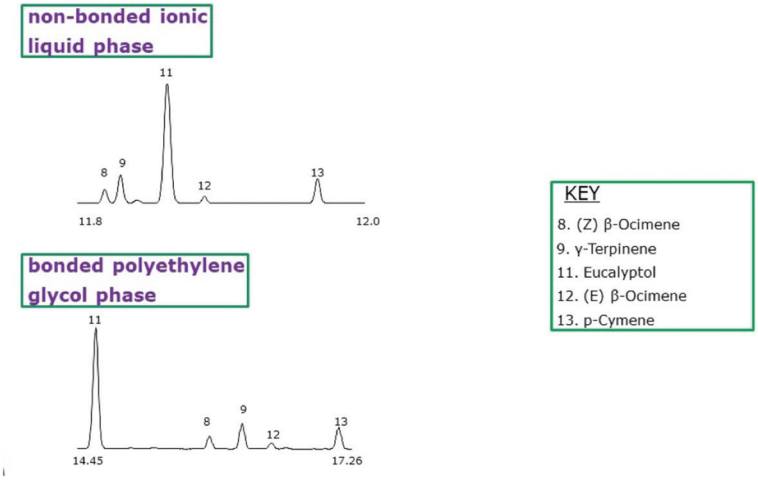

Figure 6: Phase Comparison of Farwest Native Spearmint Oil Natural Enlargement.

Conclusion

Due to the complexity of essential oils, it is important to know the chemical composition and establish a fingerprint elution profile for each essential oil. Knowing the chemical profile helps identify the essential oil and can rule out adulteration with routine testing using a primary column (SLB®-IL60i) and a confirmation column (bonded-polyethylene glycol phase). Figures 1 and 4 illustrate that the ionic liquid phase has advantages over the bonded polyethylene glycol (Figures 2 and 5). Tables 1 and 2 summarise the comparison of Willamette Peppermint Oil and Farwest Native Spearmint Oil employing two different polar phases. The ionic liquid phase offers better separation, faster retention time, and a higher maximum temperature with lower column bleed. The PEG phase has active hydroxyl (-OH) groups at the polymer termini that make these phases susceptible to a back-biting reaction if exposed to moisture and oxygen, leading to phase degradation, and contributing to column bleed. A major limitation of the PEG phases is their thermal limit of around 280°C. These drawbacks of the PEG stationary phase make the ionic liquid phase an excellent alternative.

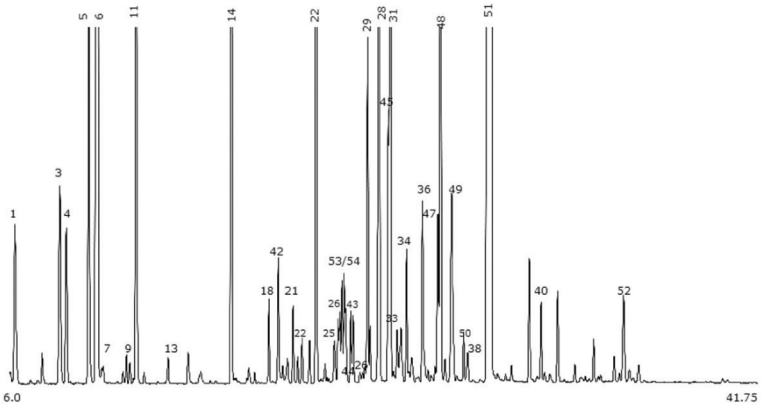

Figure 7: Farwest Scotch Spearmint Oil, ionic liquid column.

Table 2: Comparison of the main components of Farwest Native Spearmint Oil Natural using two different polar phases.

Comparison of Capillary Phases	Ionic liquid Phase	Bonded Polyethylene Glycol Phase
Retention Time	~39.37 minutes	~44.796 minutes
Separation of Four Major Components in Spearmint Oil ((Z) β-ocimene (pk 8), γ-terpinene (pk 9), eucalyptol (pk 11), (E) β- ocimene (pk 12))	Baseline separation of all four components	Baseline separation of all four components
Baseline	No baseline rise (maximum temperature 280 °C)	Baseline rise present (maximum temperature 280 °C)
Unique Separations	Eucalyptol pk 11 Elution Order: Peaks 8,9,11,12	Eucalyptol pk 11 Elution order: Peaks 11,8,9,12

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