Automatic Injection Solid-Phase Microextraction–Chiral-Gas Chromatography–Mass Selective Detection Analyses of Essential Oils

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Abstract

A viable approach for the analysis of the enantiomeric distributions of volatile components in essential oils based on automatic injection solid-phase microextraction–chiral-gas chromatography–mass selective detection (AutoSPME–chiral-GC–MSD) is demonstrated. From a selection of polar and non-polar fibers of varying thickness, the relatively non-polar thin-film polydimethylsiloxane fiber is found to be the best fiber for the analysis. Fiber exposure to the headspace above sub-microliter quantities of the oils for approximately 6 s is all that is required to obtain both the required sensitivity and resolution to produce excellent reproducibilities (generally ≤ 5.0%). The findings using this approach are in good agreement with previous findings based on chiral-GC–flame ionization detection analysis of solutions of essential oils. These results clearly establish AutoSPME as a simple, rapid, accurate, and precise method of sample preparation for the qualitative and quantitative analysis of enantiomeric distributions within essential oils. This work will be expanded to include studies directed at determining the purity of additional essential oils as well as experiments directed at determining the production location of essential oils.

Introduction

The issue most often encountered when dealing with flavor and fragrance research is the chiral nature of the components of interest. The origin of the chiral distributions of the essential oil components rests with the biogenic formation mechanisms of the specific plants. Thus, in some cases, one chiral isomer may be present, but its enantiomer is absent. On other occasions, both the dextrorotatory (*d*,+) and levorotatory (*l*,–) isomers can be found in the essential oil isolate. The distribution of the positive or negative isomers is of critical importance, because the intensity of the flavor or fragrance compound is often related to the stereochemistry of the component (i.e., *d* or *l*).

Knowledge of the distribution of enantiomers in essential oils has been greatly facilitated by the development of stable capillary gas chromatographic (GC) columns that have the capacity to resolve the enantiomers of interest (1–7). The phase in the vast majority of these capillary columns is based on cyclodextrin technologies. Through the combination of normal phase and chiral phase using multidimensional GC, enantiomeric pairs have been well resolved and identified (8–10).

The conventional approaches to essential oil sample preparation have involved the preparation of dilute solutions of the essential oil in a relatively volatile organic solvent such as methylene chloride or chloroform. In a more recent development, manual solid-phase microextraction (SPME) was reported to provide excellent qualitative and semiquantitative analyses of the volatile components of a cedarwood essential oil with no accompanying sample dissolution in an organic solvent (11). SPME is a relatively new, solvent-free method of sample preparation that involves analyte adsorption followed by the thermal desorption of analytes, usually within the heated injection port of a GC. With recent advances in SPME technology, the SPME approach has been automated for the trace analysis of organic compounds in aqueous samples (12). SPME has also been applied to the analysis of flavors (13). To date, however, no report has appeared describing the linking of automated SPME (AutoSPME) with the separation of optical isomers in essential oils. Specifically, this report describes the first AutoSPME–chiral-GC–mass selective detection (MSD) analysis of the headspace above essential oils. In addition, the isomer distribution for selected volatile components is disclosed and compared with the results from previous findings. AutoSPME appears to have the potential to provide a rapid, precise, accurate, convenient, and solvent-free extraction procedure for the quantitative analysis of optical isomer distributions in essential oils.

Experimental

Instruments

The AutoSPME–chiral-GC–MSD analyses were performed using the following equipment: a Varian (Walnut Creek, CA) 8200 vibrating SPME III autosampler fitted with a selected SPME fiber from Supelco (Bellefonte, PA) mounted atop a Hewlett-Packard (Palo Alto, CA) 5890 GC. The GC was fitted with a Restek

Corporation (Bellefonte, PA) Rt-β-DEXsm capillary column (30 m × 0.25-mm i.d., 0.25-µm film thickness). The back pressure on the column was 20 psi, and the AutoSPME injections were operated in the split mode with a split ratio of approximately 10:1. The fibers were exposed to the headspace of the samples of interest for 0.1 min with vibration prior to injection. The GC oven temperature was held at an initial value of 40°C for 1 min, then programmed to 160°C at 2°C/min, and the oven was held at 160°C for 3 min. The GC injection port and MSD interface were held at 230°C.

The SPME fibers were activated, stored, and handled strictly following the manufacturer's instructions.

Reagents

Grapefruit (#2369) and lavender (#1574) oils were received from Charabot (Grasse, France) and used as received. All reagents were obtained from Aldrich (Milwaukee, WI) with the exception of the carvone enantiomers, which were obtained from Acros (Pittsburgh, PA). All standard enantiomers were used as received.

Sample preparation

Approximately 0.5 mg of the solid or liquid sample of interest was added to a 2-mL screw-top clear vial with a hole cap and PTFE/Silicone septum (Supelco). The vial was sealed and placed in the autosampler "puck". The headspace above the essential oil was sampled as previously described.

Data collection and analysis

The operation of the AutoSPME autoinjector/tower was controlled using software provided by Varian. The Varian software was also employed to activate the automated collection of mass spectral data using the mass spectral data collection software provided by Hewlett-Packard. A minimum of six injections of individual vials was performed to obtain representative sampling of the essential oil headspace.

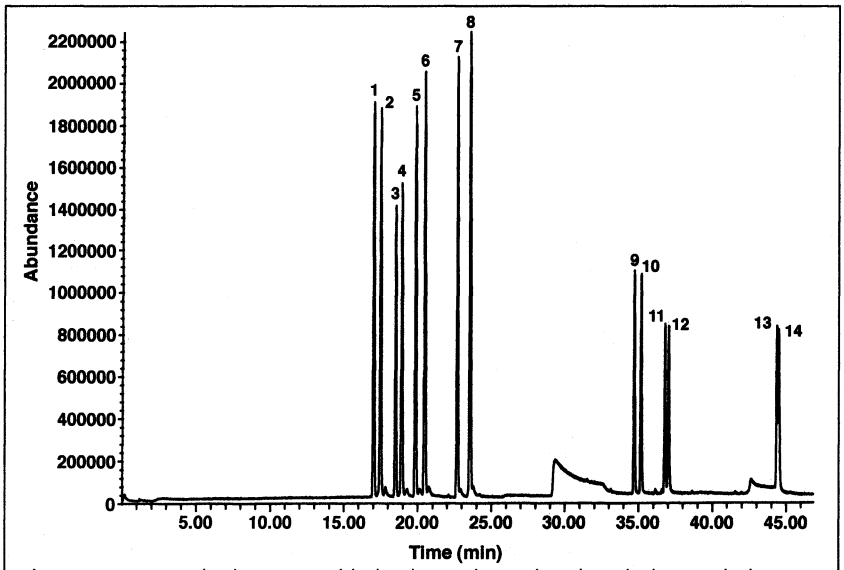

Figure 1. AutoSPME–chiral-GC–MSD of the headspace above selected standard essential oil components. Peaks: 1, (–)-α-pinene; 2, (+)-α-pinene; 3, (–)-camphene; 4, (+)-camphene; 5, (+)-β-pinene; 6, (–)-β-pinene; 7, (–)-limonene; 8, (+)-limonene; 9, (–)-linalool; 10, (+)-linalool; 11, (–)-camphor; 12, (+)-camphor; 13, (+)-carvone; 14, (–)-carvone.

Compound identification was facilitated with the use of GC retention time data bases and mass spectral search results from the Wiley (New York, NY) and NBS (National Bureau of Standards, Gaithersburg, MD) libraries of mass spectral data.

Results and Discussion

The structure and amount of volatile, semivolatile, and nonvolatile components in a wide, diverse array of essential oils has been well documented over the years. The research efforts surrounding the research on essential oils have included an array of instrumental analyses involving GC and liquid chromatography with a variety of detectors including flame ionization detection (FID) and mass spectrometry. The identity and quantitative distribution of the volatile components of cold-pressed grapefruit oil have been known for some time (14–16). The volatile components of the oil are dominated by terpene hydrocarbons, specifically (+)-limonene, and by alcohols, specifically linalool, whereas approximately 7.5% (by weight) of the oil is classified as nonvolatile. The quantitative assessments of the distributions of the enantiomers in essential oils, such as grapefruit, have been greatly aided through the development of GC column phases capable of separating enantiomers. The AutoSPME–chiral-GC–MSD analysis described here combines the effectiveness of the chiral separation capabilities of cyclodextrin phases with the automated solventless sampling capability of the AutoSPME injector and MSD to yield a novel approach to the analysis of essential oils. The effectiveness of the approach is a result of the adsorption of the volatile components of an essential oil onto the selected SPME fiber followed by thermal desorption within the confines of a heated GC injection port.

During the development of this analytical approach, various SPME fibers were examined for their capability to adequately adsorb selected volatile essential oils components. A wide array of both fiber thickness and fiber type were extensively evaluated. In the final assessment, the relatively thin (7-µm film thickness), relatively non-polar polydimethylsiloxane (PDMS) fiber was selected based on the net amount of volatile components adsorbed onto the fiber and the reproducibility of the adsorption. The relatively polar carboxenpolydimethylsiloxane fiber was found to possess a measurable memory phenomenon; that is, a blank response was difficult to obtain after the initial exposure to the essential oil or pure components. The relatively polar carbowaxdivinylbenzene (CWDVB) fibers were not as effective as the 7-µm PDMS fiber in adsorbing the broad array of compounds. For example, only trace amounts of the more-volatile, early-eluting, low-molecular-weight enantiomers were extracted from the headspace above the oils by the CWDVB fiber. Also, the SPME fiber with a thicker film (100-µm) was found to adsorb significantly more volatile components than its 7-µm counterpart, which led to

column overload and unacceptable band broadening.

The PDMS SPME fiber with a 7-µm film thickness, in combination with chiral-GC–MSD, proved to be capable of separating standard essential oil components and materials, such as the grapefruit components limonene and linalool. The data revealed that the approach had excellent sensitivity for the volatile components and excellent chromatographic resolution of the enantiomers (Figure 1). Note the baseline or near-baseline resolution of all of the standard enantiomers examined (with the sole exception of the carvone enantiomers).

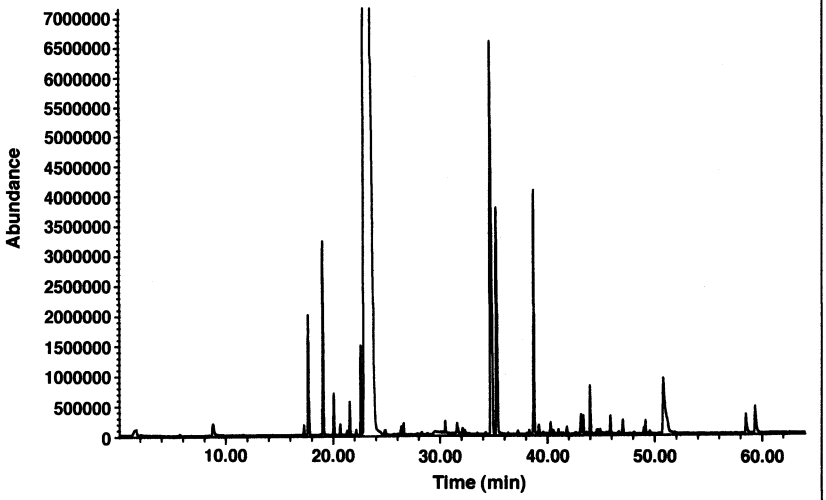

Figure 2. AutoSPME–chiral-GC–MSD total ion chromatogram of the headspace above grapefruit oil.

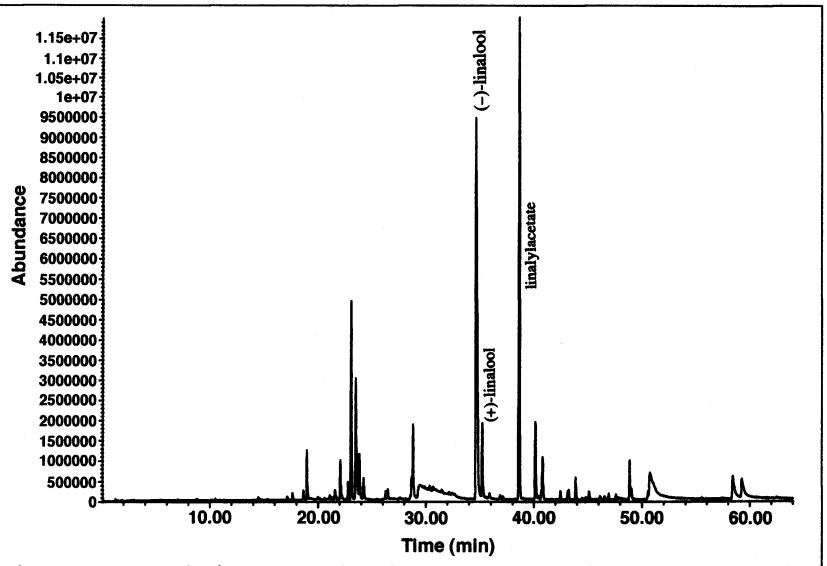

Figure 3. AutoSPME–chiral-GC–MSD total ion chromatogram of the headspace above lavender oil.

Previous experiments with manual SPME have indicated that the approach possesses excellent reproducibility (11,17,18). To gain an appreciation of the reproducibility of the AutoSPME approach, the average enantiomeric ratios of selected volatile grapefruit oil components (Figure 2) determined from injections of six individual vials, each containing ~0.5 µL of grapefruit oil, were collected (Table I). The approach yielded data with minimal variation, as depicted by the percent relative standard deviation (%RSD) values. The %RSD values were calculated by dividing the standard deviation of the six replicates by the average of the six replicates and multiplying this value by 100. This very acceptable reproducibility was in good agreement with SPME findings using a manual approach (11,17,18). In addition, the reproducibility was very comparable to recent findings of the AutoSPME analysis of organic compounds at trace levels in water (12). The percent distribution of optical isomers was calculated by dividing the area of one isomer by the area sum of both isomers times 100. Thus, in every case, the calculated isomer percentage totalled 100%.

The high enantiomeric purity of selected essential oils has been clearly established. For such essential oils as lavender oils, the enantiomeric purity has been established by the high concentration of *R*-(–)-linalool and *R*-(–)-linalyl acetate relative to their *S* enantiomer counterparts (19–21). Based on analysis by chiral-GC–FID, higher amounts of *S*-(+)-linalool in lavender essential oils have been interpreted as resulting from either a blend with synthetic racemates or the hydrolysis of linalyl acetate during the distillation (19–21). To gain an appreciation of the capability of the AutoSPME approach to determine the enantiomeric purity of a lavender oil, six injections of the headspace above a lavender oil were performed, and the distribution

Table I. Optical Isomer Distributions in Grapefruit E Oil by AutoSPME

Isomer	Retention time (min)							Mean ratio	Standard deviation	%RSD
(–)-α-pinene	17.26	6.02	6.60	6.54	5.85	5.95	5.65	6.10	0.38	6.30
(+)-α-pinene	17.59	93.98	93.40	93.46	94.15	94.05	94.35	93.90	0.38	0.41
(+)-β-pinene	20.05	80.54	80.73	81.04	80.39	80.24	80.89	80.64	0.30	0.38
(–)-β-pinene	20.65	19.46	19.27	18.96	19.61	19.76	19.11	19.36	0.30	1.57
(–)-limonene	22.52	1.02	1.00	1.00	1.00	0.99	0.97	1.00	0.02	1.72
(+)-limonene	22.82	98.98	99.00	99.00	99.00	99.01	99.03	99.00	0.02	0.02
(–)-linalool	34.55	67.78	68.53	67.95	67.38	67.97	67.56	67.86	0.40	0.58
(+)-linalool	35.12	32.22	31.47	32.05	32.62	32.03	32.44	32.14	0.40	1.24

of the linalool enantiomers was determined (Figure 3). The data clearly identify this lavender oil as authentic. For example, the *R*-(–)-linalool was found to be the dominant enantiomer, accounting for 89.17% of the total linalool. This finding is in agreement with the report that higher amounts of *S*-(+)-linalool (> 15%) can be interpreted as resulting from a blend with synthetic racemates or poor processing conditions during oil distillation (22). As would be somewhat expected because of the relatively low vapor pressure, the AutoSPME approach did not indicate the presence of the higher molecular weight components shown to be present in essential oils such as lavender.

In 1990, Mosandl et al. (21) used a combination of heartcutting GC and enantiomeric analysis by chiral GC to examine the enantiomer ratio of the optical isomers of α-pinene, β-pinene, and limonene in grapefruit oil. The results of their analysis are summarized as follows: α-pinene (0.6%)-(1*R*)-(+)-(99–100%), (1S)-(–)-(trace); β-pinene (0.05%)-(1*R*)-(+)-(63–66%), (1*S*)-(–)-(34–37%); and limonene (75.2–96%)4*R*-(+)-(100%), (4*S*)-(–)-(trace). The values for the optical isomer distributions in grapefruit oil compare very favorably with those found in the present work (Table I).

Conclusion

AutoSPME–chiral-GC–MSD has been shown to be a viable approach for the analysis of the enantiomeric distributions of volatile components in essential oils. The relatively non-polar, thin-film, 7-µm PDMS fiber was found to be the best fiber for the analysis. Short exposure times of approximately 6 s were all that was required to obtain both the required sensitivity and resolution to afford analyses with excellent reproducibilities (< 5.0%). The rapid analysis time, precision, accuracy, and lack of any sample preparation demonstrated here make AutoSPME–chiral-GC–MSD a viable approach for the qualitative and quantitative determination of optical isomer distributions within essential oils. The results presented here are in good agreement with those previously found from chiral-GC analysis of solutions of essential oils. This work will be expanded to include studies directed at determining the purity of addition essential oils as well as experiments directed at determining the production location of essential oils.

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