Examination of the Enantiomeric Distribution of Certain Monoterpene Hydrocarbons in Selected Essential Oils by Automated Solid-Phase Microextraction–Chiral Gas Chromatography–Mass Selective Detection

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Abstract

A viable approach for the determination of sources of essential oils based on automatic injection solid-phase microextraction–chiral-gas chromatography–mass selective detection is demonstrated. With no sample preparation, it is shown that the source of essential oils such as peppermint, spearmint, and rosemary can be easily distinguished. Short fiber exposure times of approximately 6 s to the headspace above submicroliter quantities of the selected oils are all that is required to obtain both the required sensitivity and resolution to afford analyses with excellent reproducibilities (relative standard deviation values consistently less than 5.0%).

Introduction

The origin of the chiral distributions of the essential oil components rests with the genetically controlled biosynthetic mechanisms of the specific plants. Thus, in some cases, one chiral isomer may be present to the exclusion of its enantiomer. On other occasions, both the dextrorotatory (*d*,+) and levorotatory (*l*,–) isomers can be found in the essential oil. The distribution of the +/– isomers is of critical importance, because the intensity of the chiral flavor or fragrance compound is related to its stereochemistry (i.e., *d* or *l*).

The development of stable capillary gas chromatographic (GC) columns, which have the capacity to resolve the enantiomers of interest (1–7), has increased the knowledge of the distribution of enantiomers in essential oils. The phase in the vast majority of these capillary columns is based on cyclodextrin technologies. Through the combination of normal-phase and chiral-phase column technologies coupled with multidimensional GC, enantiomeric pairs in essential oils have been well resolved, identified, and quantitated (8).

The conventional approach to essential oil sample preparation prior to analysis by GC involves the making up of a relatively dilute solution of the oil in a volatile organic solvent such as methylene chloride or chloroform. In a more recent development, manual solid-phase microextraction (SPME) was reported to provide for excellent qualitative and semiquantitative analyses of the volatile components of a Virginia cedarwood oil (9). 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. Recent advances in SPME technology have appeared wherein the SPME approach has been automated for the trace analysis of organic compounds in aqueous samples (10). SPME has also been applied to the analysis of flavors (11). Very recently, the first report describing the linking of automated SPME (AUTOSPME) with chiral gas chromatography (chiral-GC) and mass selective detection (MSD) for the separation of optical isomers in essential oils has appeared (12). The findings in this recent work revealed that the approach was viable for the qualitative and quantitative analysis of the enantiomeric distribution of components in essential oils.

The determination of the enantiomeric distribution of chiral compounds in essential oils has become quintessential in determining the origin and authenticity of oils (1,13–21). This report generally employs the AUTOSPME approach described earlier (12) with a focus on examining the potential of the approach to ascertain the origin of an array of essential oils. In addition, the optical isomer distribution for selected volatile components of the oils is disclosed and compared with results from previous findings through alternative approaches.

Experimental

Instrumental

The AUTOSPME–chiral-GC–MSD analyses were performed using the following equipment: a Varian Instruments (Walnut Creek, CA) 8200 vibrating SPME III autosampler fitted with a

7-µm polydimethylsiloxane (PDMS) SPME fiber from Supelco (Bellefonte, PA) was mounted atop a Hewlett-Packard (Palo Alto, CA) 5890 GC. The GC was fitted with a Restek Corporation (Bellefonte, PA) Rt-beta-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. 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. 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.

Essential oil sources

Peppermint oils were obtained from Argentina (Industrial J. Matas, Villaneuva Mendoza, Argentina); Australia (Essential Oils of Tasmania, Hobart, Australia); Bulgaria(1), France(1), Morocco(1), Spain, and former USSR (Haarman & Reimer, Holzminden, Germany); Canadian (Pan Oil, Bow Island, Canada); Croatia (D. Kustrak, Zagreb, Croatia); England (Wm. Ransom, Hitchin, U.K.); France(2) (INRA, Antibes, France); Indian Badaun and India Punjab (Jindal Dye Intermediate, NY); Italy (Anthess, Pancalieri, Italy); Morocco(2) (B. Benjilali, Rabat, Morocco); New Zealand (M. F. Barnes, Canterberry, New Zealand); Poland (Pollena Aroma, Warsaw, Poland); USA-Madras, Midwest, Montana, Ontario (E. Idaho); Willamette and Yakima (Wm. Leman, Bremen, U.S.A.); and Yugolsalvia (O. Gasic, Novi Sad, Yugoslavia).

Native spearmint oils were obtained from France (Charabot, Grasse, France) and Farwest-USA (Wm. Leaman, Bremen, U.S.A.).

Scotch spearmint oils were obtained from Farwest-USA (Wm. Leman, Bremen, U.S.A.), Canadian(1) (Wm. Leman, Bremen, U.S.A.), and Canadian(2) (Pan Oil, Bow Island, Canada).

Rosemary oils were obtained from Morocco(1) (Charabot, Grasse, France); Morocco(2) (Berjé Bloomfield, NJ, U.S.A.); Morocco(3) (Citrus & Allied, Lake Success, NY, U.S.A.); Morocco(4), Tunisia(1), and Spain(1) (H.E. Daniel, Royal Tunbridge Wells, England); Algeria(1–5) (B. Bellomari, Camerino, Italy); Italy(1–4) (M. Moretti, Sassari, Italy); Spain(2–3) (A. Velasco-Negueruela, Madrid, Spain); Slovakia (I. Solomon, Michehalovice, Slovakia); Hungary (J. Domokis, Budapest, Hungary); and Tunisia(2–3), Australia(1–3), Australia(4) oil of alba cultivar, Australia(5) oil of Majorca pink cultivar, Australia(6) oil of prostratus cultivar, Australia(7) oil of rosea cultivar, and Australia(8) oil of Tuscan blue cultivar (L. Doimo, Australian Tea Tree Oil Research Institute, Lismore, Australia).

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 poly(tetrafluoroethylene)/silicone septum (Supelco, Bellefonte, PA). The vial was sealed and placed in the Varian AUTOSPME autosampler puck. The headspace above the oil was sampled as previously described.

Data collection and analysis

The operation of the AUTOSPME autoinjector/tower was controlled via software provided by Varian. The Varian software was also employed to activate the automated collection of mass spectral data via the HP-provided mass spectral data collection software. A minimum of 6 injections of individual vials was made to obtain representative sampling of the essential oil headspace.

Compound identification was facilitated through the use of GC retention time databases, authentic samples of specific enantiomers, and mass spectral search results from the Wiley and NBS libraries of mass spectral data.

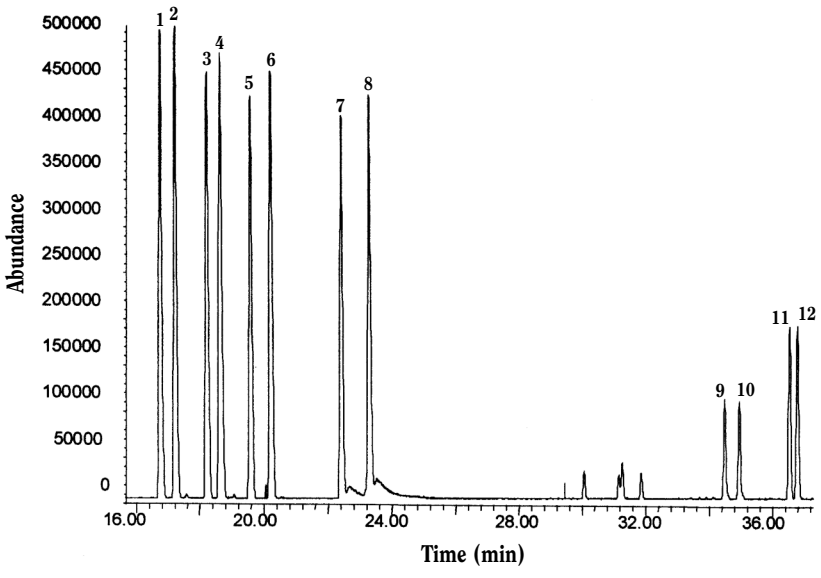

Figure 1. Total ion chromatogram from AUTOSPME–Chiral-GC–MSD analysis of selected terpenes. Peak identifications: 1, (–)-α-pinene; 2, (+)-α-pinene; 3, (–)-camphene; 4, (+)-camphene; 5, (+)-β-pinene; 6, (–)-β-pinene; 7, (–)-limonene; 8, (+)-limonene; 9, (–)-linalool; 10, (+)-linalool; 11, (–)-camphor; 12, (+)-camphor.

Results and Discussion

In an earlier report, an AUTOSPME–chiral-GC–MSD analysis (12) combined the effectiveness of the chiral separation capabilities of fused-silica capillary columns having cyclodextran phases with the automated solventless sampling capability of the AUTOSPME injector and MSD to yield a novel qualitative and quantitative approach to the analysis of the essential oils. The analytical methodology was shown to be precise and accurate. A very similar separation of selected monoterpene hydrocarbons was obtained in this study

(Figure 1). This AUTOSPME–chiral-GC–MSD approach has not been applied to the analysis of the distribution of selected chiral isomers in a series of rosemary, spearmint, and peppermint oils of different origins.

Peppermint oil

The enantiomeric distribution of the chiral isomers of α-pinene, pinene, and limonene in these commercial oils of peppermint is shown in Table I. From the data, it can be seen that there is stability in the enantiomeric distributions. It can be concluded that (–)-limonene is the predominant enantiomer found. However, it would appear that the reduced levels in the oils of Bulgaria(1), Morocco(1), New Zealand(1), Poland, and Spain could indicate some slight adulteration; however, this is not conclusive.

Spearmint oil

The enantiomeric distribution of four monoterpene hydrocarbons in Native (*Mentha spicata* L.) and Scotch (*Mentha gracilis* Sole) spearmint oils are shown in Table II. From the data, it can be seen that the distribution of these monoterpene hydrocarbons is stable for both spearmint oils, and these cannot be used to differentiate between two different botanical source oils.

Rosemary oil

The enantiomeric distribution of camphor in rosemary oil was determined by Ravid et al. (17) to be (*S*)-(–)-camphor (34%) to (*R*)-(+)-camphor (60%). More recently, these same authors (18) found that the enantiomeric distribution of borneol in rosemary oil was as shown in Table III. According to König et al. (19), rosemary oil is one of the most commonly adulterated oils on the market. These authors determined the enantiomeric distribution of borneol. They found that while the amount of (–)-borneol differed significantly, the enantiomeric excess was much larger in Spanish rosemary oil than the other oils examined. Furthermore, they found that no correlation could be found between the enantiomeric distribution of (+/–)-camphor or (+/–)-α-terpineol. In fact, the varying enantiomeric distribution of these two compounds was determined to be of no diagnostic value for authenticating rosemary oils of various origin.

The enantiomeric distribution of 6 selected constituents in 30 rosemary oils of differing sources can be seen in Table IV. With the percent relative standard deviation (%RSD) values for the determination of the isomer distributions for these samples at less than 2.5%, differentiation among oil sources was

Table I. Enantiomeric Distribution of α-Pinene, β-Pinene, and Limonene in Peppermint Oils of Different Geographic Origins

Oil origin	(–/+)-α-Pinene	(–/+)-β-Pinene	(–/+)-Limonene
Argentina	47.0/53.0	49.9/52.1	92.0/8.0
Australia	45.2/54.8	47.8/52.2	88.2/11.8
Bulgaria(1)	48.1/51.9	49.4/50.6	72.6/27.4
Bulgaria(2)	67.5/32.5	50.0/50.0	93.7/6.3
Canadian	46.8/53.2	51.4/48.6	90.8/9.2
Croatia	50.1/49.9	53.6/46.4	91.9/8.1
England	65.1/34.9	46.7/53.3	92.5/7.5
France(1)	47.7/52.3	48.9/51.1	78.4/21.6
France(2)	46.6/53.4	48.2/51.8	90.7/9.3
India, Badaun	56.4/43.6	41.7/58.3	91.9/8.1
India, Panjab	54.4/45.6	43.3/56.7	94.2/5.8
Italy	45.1/54.9	47.8/52.2	89.0/11.0
Morocco(1)	68.1/31.9	24.5/75.5	76.7/23.3
Morocco(2)	49.7/50.3	50.6/49.4	87.9/12.1
New Zealand	45.9/54.1	48.5/51.5	74.4/25.6
Poland	52.8/47.3	53.2/46.8	76.5/23.5
Spain	45.2/54.8	48.1/51.9	60.5/39.5
U.S.A., Madras	47.1/52.9	51.8/48.2	88.8/11.2
U.S.A., Midwest	46.6/53.4	52.0/48.0	89.9/10.1
U.S.A., Montana	49.1/50.9	40.6/49.3	90.7/9.3
U.S.A., Ontario	46.1/53.9	51.8/48.2	84.0/16.0
U.S.A., Willamette	47.7/52.3	51.3/48.7	85.9/14.1
U.S.A., Yakima	47.0/53.0	51.5/48.5	79.9/20.1
USSR (formerly)	68.0/32.0	48.5/51.5	96.7/3.3
Yugoslavia	50.4/49.6	46.2/53.8	98.3/1.7

Table II. Enantiomeric Distribution of α-Pinene, Camphene, β-Pinene, and Limonene in Native and Scotch Spearmint Oils of Different Origins

Oil Origin	(–/+)-α-Pinene	(–/+)-Camphene	(–/+)-β-Pinene	(–/+)-Limonene
Native spearmint				
French	62.40/37.60	< 0.1/> 99.9	52.1/47.9	98.8/1.1
Farwest USA	59.7/40.3	< 0.1/> 99.9	51.3/48.7	98.1/1.9
Scotch spearmint				
Farwest USA	63.5/36.5	< 0.1/> 99.9	52.7/47.3	99.1/0.1
Canadian(1)	64.4/35.6	< 0.1/> 99.9	46.8/53.2	99.0/1.0
Canadian(2)	62.4/37.6	< 0.1/> 99.9	54.6/45.4	99.8/0.2

Table III. Enantiomeric Distribution of Borneol in Cultivars of Rosemary*

Cultivar	Percentage	(1*R*)-(+)-Borneol	(1*S*)-(–)-Borneol
Israel(1)	8.4	55	45
Israel(2)	5.9	26	74
Greece	6.2	43	57
Spain	9.4	33	67
"Majorca"	0.8	4	96
"Corsican"	2.6	83	17
"Tuscan blue"	11.2	5	95
"Seven sea"	1.2	16	84
"Frimley blue"	1.3	83	17
"Prostrale"	6.1	62	27

* According to Ravid et al. (17).

possible. Examination of the data reveals that the enantiomers of borneol were not found in each oil. We did not attempt to determine the enantiomeric distribution of a component if it existed in a level of < 0.5%. Of the samples examined, the Moroccan and Tunisian oil samples were of commercial origin, as were the oils of Spain(1) and Australia(1–3). Australia(4–8) oils were produced from horticultural cultivars of rosemary and were not from plants grown to specifically produce oils. All of the Algerian and Italian oils and the oils of Spain(2) and Spain(3) were oils produced in the lab from plants collected in the wild. The oils from Slovakia and Hungary were also produced in the lab, although they were from plants grown in experimental gardens.

From the data presented in Table IV, it can be seen that the enantiomeric distribution of the selected components of all of the Algerian oils was very stable. Similar stability in enantiomeric distribution can be seen in the oils of Spain(2), Spain(3), Italy (1), Italy(2), and Italy(3). The low level of limonene in the oil of Italy(4) appears to be a natural phenomenon.

Examination of the data obtained for the commercial oils reveals that some enantiomeric distribution differences could be found. In particular, it is evident to us that the oils of Morocco(4) and Tunisia(2) were adulterated with (+)-limonene. The oil of Spain(1) also appeared to be adulterated with a coupage of (+)-α-pinene, β-pinene, and (+)-limonene.

These results from the AUTOSPME–chiral-GC–MSD were very similar to those of Kreis et al. (20), who employed a multidimensional GC–flame ionization detection approach. Some of the advantages of this approach, in comparison with the method of Kreis et al., include (*a*) simpler instrumentation, (*b*) absence of solvents, (*c*) faster separations, and (*d*) positive compound identification via mass spectra. However, both methods provide powerful analytical tools for the investigation of origin and authenticity of essential oils.

Conclusion

AUTOSPME–chiral-GC–MSD has been shown to be a viable approach for the speciation of the origin of essential oils. Short fiber exposure times of approximately 6 s were all that was necessary to obtain both the required sensitivity and resolution to afford analyses with excellent reproducibilities (RSD < 5.0%). The minimal sample preparation, rapid analysis time, accuracy, and precision demonstrated here make AUTOSPME–chiral-GC–MSD a viable approach for the determination of the essential oil origins. The results presented here are in direct concert with those previously found from other chiral-GC

Table IV. Enantiomeric Distribution of Selected Components of Rosemary Oil of Different Origins

	(–/+)-α-Pinene		(–/+)-Camphene		(–/+)-β-Pinene		(–/+)-Limonene		(–/+)-Camphor		(–/+)-Borneol	
Morocco(1)	43.9	56.1	65.8	34.2	75.3	24.7	51.0	49.0	30.7	69.3	69.3	30.7
Morocco(2)	42.8	57.2	59.1	40.9	72.5	27.5	57.8	42.2	24.0	76.0	66.0	34.0
Morocco(3)	40.0	60.0	60.3	39.7	73.6	26.4	53.2	46.8	17.1	82.9	48.1	51.9
Morocco(4)	36.8	63.2	57.3	42.7	74.1	25.9	2.1	97.9	32.6	67.4	–	–
Morocco(5)	38.6	61.4	56.4	43.6	72.2	27.8	55.9	44.1	22.2	77.8	68.9	31.1
Algeria(1)	84.5	15.5	93.0	7.0	92.2	7.8	82.8	17.2	81.0	19.0	–	–
Algeria(2)	78.5	21.5	92.1	7.9	89.4	10.6	85.5	16.5	79.5	20.5	–	–
Algeria(3)	82.3	17.7	93.5	6.5	90.5	9.5	85.4	14.6	79.7	20.3	–	–
Algeria(4)	81.0	19.0	92.5	7.5	92.9	7.1	81.8	18.2	74.8	25.2	–	–
Algeria(5)	85.1	14.9	93.2	6.8	93.9	6.1	82.9	17.1	79.7	20.3	–	–
Tunisia(1)	41.7	58.3	62.8	37.2	74.7	25.3	56.3	43.7	17.1	82.9	44.1	55.9
Tunisia(2)	43.6	56.4	66.2	33.8	75.4	24.6	2.8	97.2	18.5	81.5	–	–
Tunisia(3)	52.1	47.9	48.7	51.3	82.1	17.9	37.3	62.7	34.6	65.4	79.1	20.9
Spain(1)	16.4	83.6	54.0	46.0	90.8	9.2	20.8	73.2	47.9	52.1	–	–
Spain(2)	37.2	62.8	75.5	24.5	78.9	21.1	59.4	40.6	42.5	57.5	–	–
Spain(3)	35.6	64.4	74.6	25.4	79.7	20.3	63.4	36.6	41.4	58.6	–	–
Italy(1)	13.1	86.9	65.0	35.0	73.2	26.8	53.0	47.0	56.5	43.5	–	–
Italy(2)	8.0	92.0	62.1	37.9	74.0	26.0	51.7	48.3	69.3	30.7	–	–
Italy(3)	7.2	92.8	55.1	44.9	29.5	70.5	52.3	47.7	27.5	72.5	–	–
Italy(4)	7.6	92.4	55.9	44.1	66.1	33.9	–	–	79.1	20.9	–	–
Slovakia	70.5	29.5	41.0	59.0	92.4	7.6	53.2	46.8	48.4	51.6	58.0	42.0
Hungary	8.7	91.3	53.5	46.5	91.2	8.8	30.2	69.8	49.0	51.0	78.4	21.6
Australia(1)	16.9	83.1	61.7	38.3	56.5	43.5	37.2	62.8	72.8	27.2	86.3	13.7
Australia(2)	16.0	84.0	52.3	47.7	61.3	38.7	51.7	48.3	73.3	26.7	80.8	19.2
Australia(3)	50.2	49.8	53.9	46.1	79.4	20.6	52.2	47.8	25.6	74.4	53.7	46.3
Australia(4)	54.8	45.2	65.9	34.1	84.1	15.9	54.0	46.0	26.9	73.1	67.4	32.6
Australia(5)	24.5	75.5	14.0	86.0	85.1	14.9	50.1	49.9	26.3	73.7	47.7	52.3
Australia(6)	33.8	66.2	54.7	45.4	72.9	27.1	48.6	51.4	11.2	88.8	68.6	31.4
Australia(7)	17.5	82.5	49.9	50.1	81.0	19.0	52.0	48.0	10.8	89.2	50.1	49.9
Australia(8)	54.1	45.9	52.5	47.5	81.1	18.0	39.2	60.8	34.6	65.4	93.8	6.2

analyses of solutions of oils involving more complex instrumentation and sample preparation. This work will be expanded to include studies directed at determining the genuineness of essential oils as well as experiments directed at determining the production location of essential oils.

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