SUPPLEMENTARY MATERIAL

Fast analysis of volatile compounds from *Lippia citriodora* with nanoporous aluminum wire as solid-phase microextraction fibres

Mohammad Nemati Kharajuo^a, Marzieh Piryaeei^{b*}, Marzieh Babashpour Asl^a, Mir Mahdi Abolghasemi^b

^aDepartment of Horticulture, College of Agriculture, Maragheh Branch, Islamic Azad University, Maragheh, Iran.

^bDepartment of Chemistry, Faculty of Science, University of Maragheh, Maragheh, Iran

* Corresponding author:

Marzieh piryaeei

Tel: +98 411 6689859; Fax: +98 411 6689859

E-mail address: m.piryaeei@gmail.com

Abstract

In this study, the efficiency of nanoporous on aluminum wires as fibers for headspace solid-phase microextraction (HS-SPME) of volatile compounds from *Lippia citriodora* were investigated and compared with two anodized methods. The prepared fibers are durable with very good chemical and thermal stability which can be coupled to GC and GC/MS. A one at-a-time optimization strategy was applied for optimizing the important extraction parameters such as extraction temperature, extraction time, sample mass and added water. Compared with hydrodistillation(HD), HS-SPME, provide the advantages of a small amount of sample, timesaving, simplicity and cheapness.

Keywords: nanoporous aluminum, *Lippia citriodora*, microextraction

Experimental

Plant material

The aerial parts of *Lippia citriodora* were harvested during the flowering season from North West of Iran. The plant materials were dried in air and stored in sealed bags in a cool place. A voucher specimen was deposited at the chemistry herbarium of this laboratory under the code 1205 LC.

Chemicals

Helium, 99.999%, was purchased from Roham Gas Co. (Tehran, Iran). Double distilled water was used throughout. All chemicals were obtained from E. Merck.

Apparatus

A Hewlett-Packard Agilent 7890A series GC equipped with a split/splitless injector and an Agilent 5975C mass-selective detector system were used for determination. The MS was operated in the EI mode (70 eV). Helium (99.999%) was employed as a carrier gas, and its flow rate was adjusted to 1.1 mL min⁻¹. The separation of PAHs was performed on a 30 m×0.25 mm HP-5 MS column with 0.25 µm film thickness. The column was held at 50°C and increased to 180°C at a rate of 15°C min⁻¹ and then raised to 260°C at 20°C min⁻¹ and kept at this temperature for 5 min. The injector temperature was set at 260°C, and all injections were carried out on the splitless mode for 2 min. The GC–MS interface, ion source and quadrupole temperatures were set at 280, 230 and 150°C, respectively. Compounds were identified using the Wiley 7N (Wiley, New York, NY, USA) Mass Spectral Library. GC–MS was tuned before each analysis with HP Chem-Station Standard Spectra Auto tune routine with perfluorotributylamine (PFTBA). A homemade SPME device was used for holding and the injection of the proposed fiber into the GC–MS injection port. Before its use for SPME experiment, the fiber was heated at 100°C for 20 min in oven, and finally conditioned at 260°C in a GC injection port under helium gas for 2 h until a clean blank was obtained. Moreover, blanks were run periodically during the analysis to confirm the absence of contaminants.

Hydrodistillation procedure

Air-dried aerial parts of *Lippia citriodora* (100g) were ground and subjected to hydrodistillation for 2 h, using a Clevenger-type apparatus as recommended by British Pharmacopeia. Briefly, the plant was immersed in water and heated to boiling, after

which the essential oil was evaporated together with water vapour and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate. The dried oil was stored at 4°C until analysis by GC–MS. Analysis of volatile compounds was carried out by direct injection 0.3 µL of the dried essential oil into the GC–MS.

Preparation of nanoporous aluminum SPME fibers

A roll of aluminum wire of 0.3-mm diameter with high purity was obtained from the Sim-Kat factory (Tabriz, Iran). The aluminum wire was cut into lengths of 3 cm. The cut pieces were washed in dilute detergent solution and distilled water, in turns.

These wires were used in three forms: (a) wire oxidized in air, (b) the aluminum wire was anodized in 16% (w/v) sulfuric acid solution at a controlled potential of 15 V, using an aluminum plate as the cathode, for 60 min. and (c) For the preparation of porous aluminum anodized wire, first electrochemically polished using a mixture 15 parts of 68% nitric acid and 85 parts of 85% phosphoric acid, and 40 g L⁻¹ Na₂Cr₂O₇ at 15 V for 1 h at room temperature. The sample was washed with deionized water and secondly anodized in 0.3 M oxalic acid under 40 V for 1 h at 40 °C, and the third anodization could be conducted to improve the regularity of nonporous. On the all-electrochemical and anodizing process used a platinum electrode as cathode electrode. A thick no uniform alumina film, which was formed across the surface of Aluminum wire, was removed using a solution composed of 0.4 M H₃PO₄ and 0.2 M Na₂Cr₂O₇ at room temperature. The efficiency of extraction essential oils with different fibers were shown in Fig 1S.

The headspace SPME procedure

For HS-SPME analysis, 2.0 g of plant was transferred to a 10 mL vial. Subsequently, the vial was sealed with a rubber septum and immersed in an oil bath at fixed temperature, 70°C. The needle of the SPME device was passed through the septum and its fiber was exposed to the headspace atmosphere of plant for 30 min. Subsequently, the fiber was with drawn into the needle then introduced into the GC injection port and held there for 2 min at 260°C to completely desorb the volatile flavor compounds and then analyzed by GC–MS.

Table S1. Constituents of the oil of *Lippia citriodora*.

No	Compounds	RI ^a	(HD) Area% ^b	HS-SPME Area% ^c	HS-SPME Area% ^d
1	α - thujene	927	0.8	0.5	1.2
2	α -pinene	933	1.1	1.3	0.9
3	Sabinene	972	2.3	1.7	1.9
4	β -Pinene	975	0.6	0.5	0.3
5	1- octen-3-ol	982	0.5	0.3	0.8
6	Myrcene	993	0.3	0.6	0.5
7	p-Cymene	1026	0.9	0.2	0.5
8	Limonene	1033	6.8	5.2	4.5
9	1,8-cineole	1035	0.5	0.3	0.6
10	E- β -Ocimene	1051	0.4	0.8	0.5
11	cis-sabinene hydrate	1071	0.6	0.5	0.3
12	γ - Terpinene	1061	0.5	0.2	0.5
13	linalool	1102	2.2	1.7	2.5
14	Trans-Rose oxide	1127	0.9	0.3	0.2
15	Trans-limonene oxide	1135	0.3	0.5	0.5
16	Camphor	1143	2.1	2.6	2.1
17	Terpinen-4-ol	1174	1.1	1.5	0.8
18	Borneol	1181	0.1	0.0	0.0
19	α – terpineol	1190	1.3	1.1	1.5
20	Neral	1237	14.8	15.1	14.7

21	Geranio	1255	23.2	22.3	22.4
22	Linalool acetat	1257	0.1	0.0	0.0
23	Geranial	1271	0.3	0.0	0.1
24	isopulegol acetate	1273	0.5	0.1	0.3
25	α –Terpinen-7-al	1282	0.1	0.0	0.0
26	δ -Elemene	1342	0.6	0.5	0.2
27	Eugenol	1360	1.9	1.2	1.5
28	α –Copaene	1375	0.5	0.2	0.3
29	Geranyl acetate	1379	0.3	0.2	0.3
30	α –cedrene	1411	0.5	0	0.1
31	Caryophyllene	1415	1.1	1.0	0.9
32	Cedrane	1436	0.4	0.1	0.5
33	aromadendrene	1441	0.6	0.3	0.3
34	Humulene	1447	1.7	0.6	1.2
35	Allo-Aromadendrene	1465	2.2	1.4	2.5
36	Germacrene D	1472	6.5	1.8	3.4
37	Bicyclogermacrene	1491	3.2	2.5	3.5
38	γ -cadinene	1510	0.1	0.4	0.6
39	δ -cadinene	1515	0.8	0.5	0.4
40	(E)-Nerolidol	1565	3.7	2.4	3.1
41	Spathulenol	1567	4.1	4.6	3.8
42	Caryophyllen oxide	1570	3.5	2.6	2.5
43	δ -cadinol	1611	0.3	0.2	0.1

- a) Retention indices using a HP-5MS column (relative retention times normalize to closely eluting *n*-alkanes).
- b) Relative area (peak area relative to total peak area) for hydrodistillation method.
- c) Relative area (peak area relative to total peak area) for HS-SPME method with fiber b.
- d) Relative area (peak area relative to total peak area) for HS-SPME method with fiber c.

Figure S1. The efficiency of extraction essential oils with different fibers. Al1: pour aluminum, Al2: wire oxidized in air, Al3: fiber b, Al4: fiber c.

Figure S2. Effect of extraction temperature on the extraction efficiency for (a) fiber b, and (b) fiber c.

Figure S3. Effect of extraction time on the extraction efficiency for (a) fiber b, and (b) fiber c.

Figure S4. Effect of sample weight on the extraction efficiency for (a) fiber b, and (b) fiber c.

Figure S5. Effect of water addition on the extraction efficiency of the SPME method for (a) fiber b, and (b) fiber c.