

Allium Chemistry: Supercritical Fluid Extraction and LC–APCI–MS of Thiosulfinates and Related Compounds from Homogenates of Garlic, Onion, and Ramp. Identification in Garlic and Ramp and Synthesis of 1-Propanesulfinothioic Acid S-Allyl Ester

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Supercritical fluid (SF) extracts of homogenized garlic (*Allium sativum* L.), ramp (*A. tricoccum* Ait.), and onion (*A. cepa*) were characterized with liquid chromatography (LC) and atmospheric pressure chemical ionization mass spectrometric identification. The major thiosulfinates from garlic and ramp were readily characterized by this technique. Small quantities of ajoene, a potent antithrombotic agent, were also found in SF extracts of garlic homogenates. The profiles of onion juice extracts revealed the usual thiosulfinates, zwiebelanes, and bissulfine reported in prior studies, as well as cepaenes previously identified in extracts of onion juice through extensive isolation steps and spectroscopic methods. The presence of trace quantities of allyl compounds in onion juice and propyl compounds in garlic and ramp homogenates has been verified by LC–mass spectrometry (MS). The presence of these compounds was not readily evident in previous analyses using gas chromatography–MS with cold-on-column injection and reversed-phase or normal phase LC with UV detection.

Keywords: *Supercritical fluid extraction; liquid chromatography; mass spectrometry; LC–MS; onion, Allium cepa; garlic, Allium sativum L.; ramp, Allium tricoccum Ait.; thiosulfinates*

INTRODUCTION

Characteristic flavors of fresh onion (*Allium cepa*), garlic (*A. sativum* L.) and other *Allium* spp. are associated with thiosulfinates and related compounds formed enzymatically from odorless precursors when the plants are cut or crushed (Block, 1992). Qualitative and quantitative analyses of *Allium* organosulfur flavorants take on added significance because of reported health benefits from the consumption of fresh *Allium* spp. (Block, 1994; Lawson, 1993; Koch and Lawson, 1996) and numerous health food products that claim to duplicate these benefits in other forms. For the isolation and extraction of these flavorants, methods such as supercritical carbon dioxide (SC-CO₂) extraction and room temperature steam distillation are recommended. Methods such as gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC), as well as GC–mass spectrometry (MS), LC–MS, and SFC–MS have been used for the separation and identification of the thermally unstable thiosulfinate esters and their breakdown products. Each method has its strengths and weaknesses (Block and Calvey, 1994; Ferary *et al.*, 1996; Ferary and Auger, 1996).

Interest in supercritical fluid (SF) technology has increased because of its effectiveness in the extraction and analysis of natural products (Nguyen and Evans, 1991; Nguyen *et al.*, 1992) and concern related to the use and disposal of conventional organic solvents.

Several laboratories have employed supercritical fluid extraction (SFE) methodologies in the extraction of garlic and onion. Wagner and Breu (1989) reported on the complete decomposition of allicin and other thiosulfinates, after garlic juice was stored at room temperature for 3 h and then extracted with SC-CO₂. In contrast to this finding, we have observed essentially no change in the liquid chromatographic profile of SF extracts of aqueous homogenates of garlic that were extracted at 35 °C following storage for 10 min or 2 h at room temperature (Calvey and Block, 1997). Our observations agree with those of Lawson (1993), who has shown that the half-life of allicin at room temperature is 4 days in water. Breu and Dorsch (1994) reported that cepaenes were the dominant organosulfur compounds found in SF extracts of onion while thiosulfinates represented major constituents in the corresponding chloroform extracts. The SFE conditions employed, however, were not specified in their report. Miles and Quimby (1990) extracted garlic products with SC-CO₂ under mild conditions, and the extracts were analyzed by GC with atomic emission detection. Sinha *et al.* (1992) extracted onion with SC-CO₂ under mild conditions, and the extracts were analyzed by GC–MS. These two latter research groups used traditional GC methodologies with elevated injector temperatures in the analysis of these low-temperature extracts. The identification of those compounds that are primarily responsible for the characteristic flavor of freshly cut members of the genus *Allium* was not possible with the above methods because of thermal decomposition or artifact formation (Block, 1992). We have previously reported that onion and garlic extracts obtained by SFE using SC-CO₂ compare well with diethyl ether extracts

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analyzed by subambient on-column injection GC-MS and methylene chloride extracts analyzed by LC with UV detection, respectively (Calvey *et al.*, 1994).

In this paper we use reversed-phase LC-MS and LC tandem MS (LC-MS-MS) detection methods with atmospheric pressure chemical ionization (APCI) to characterize garlic, ramp (*A. tricoccum* Ait.), and onion SF extracts. Various organosulfur components in garlic, ramp, and onion extracts are compared with synthetic standards. Ramp or wild leek is found in moist, shady woods from New Brunswick to Minnesota and south to North Carolina. It is a perennial which gives off a strong onion odor. Banquets featuring this plant are a spring tradition in southern Appalachia (Cox, 1985). The Cherokee Indians consumed ramp leaves for colds and croup or as a spring tonic, while the warm juice was applied for earaches (Foster and Duke, 1990). The ramp has a taste similar to but stronger than that of garlic. Its edible bulbs are widely harvested and sold commercially (Nault and Gagnon, 1993). Our work constitutes the first study of the flavorants derived from ramp.

EXPERIMENTAL PROCEDURES

Materials. Fresh garlic and onion were obtained from local retail markets in the Washington, DC, area. Ramp plants were collected along the banks of the Potomac River near Washington, DC, in the spring of 1995 and 1996 and from the mountains of West Virginia in the Spring of 1996. Ramp bulbs were immediately frozen or stored in the refrigerator until extracted. LC grade solvents were obtained from J. T. Baker (Phillipsburg, NJ). SFC/SFE grade CO₂ was obtained from Air Products (Allentown, PA). Hydromatrix (a mixture of diatomaceous earth and crystalline silica) was obtained from Varian (Palo Alto, CA). Synthesis of most *Allium* organosulfur compound standards are described elsewhere (Block *et al.*, 1986, 1996b, 1997; Block and O'Connor, 1974; Block and Zhao, 1992).

LC-MS and LC-MS-MS Analysis. LC-MS data were collected by using a Finnigan TSQ 7000 (Finnigan MAT, San Jose, CA) equipped with an APCI interface, and a Hewlett Packard (Hewlett Packard, Wilmington, DE) 1050 liquid chromatograph. Chromatographic separation was achieved with a YMC J'sphere ODS-M80 column (250 × 2.0 mm i.d., S-4 μm, 80Å). A linear gradient employing water/acetonitrile as the mobile phase was pumped at 200 μL/min. Initial mobile phase composition of 70% A (95/5 water/acetonitrile), 30% B (5/95 water/acetonitrile) was held constant for 10 min. The final mobile phase composition of 40% A/60% B was reached at 25 min, followed by an isocratic period of 15 min. Ionization conditions were as follows: vaporizer, 350 °C; heated capillary, 150–200 °C; corona discharge needle, 5.0 μA. Nitrogen was employed, at a sheath gas pressure of 70 psi and auxiliary gas was on at 50 mm (65 mm flow tube, maximum flow 12 L/min), to assist in nebulization. Ions were decomposed by using argon in the collision cell at 0.8 mTorr and an optimized collision energy of -12 to -15 eV for the thiosulfonates and -55 eV for the cepaenes. Day to day instrument conditions were set based on the mass spectrum of alliin (9), such that the spectrum was the same every day.

Supercritical Fluid Extraction. *Allium* species were extracted with a PrepMaster and AccuTrap (Suprex, Inc., Pittsburgh, PA). Onion slices (30–40 g) were juiced with an Oster juice extractor (Milwaukee, WI). Whole cloves of garlic (2–4 g) or ramp bulbs (2–5 g) were homogenized in 10 mL/g of water at ambient temperature by using a Tisumizer (Tekmar, Cincinnati, OH). The solutions were allowed to stand at room temperature for 5–10 min to ensure complete enzymatic conversion to the thiosulfonates and related compounds. Hydromatrix was mixed with 15 mL of onion juice (representing ≈26 g) or garlic or ramp homogenate (representing ≈1.5 g) and placed in a 50 mL extraction vessel. The mixtures were extracted at 35 °C and 240 atm at a flow rate of 2 mL/min for ca. 30 min, resulting in 60 g of liquid CO₂

used. The effluent was trapped on glass beads at 0–1 °C and desorbed with 1 mL of methanol or methanol/water (50/50).

2-Propenesulfinyl Chloride. Under an argon atmosphere, allyl chloride (8.25 g, 0.133 mol) in ether (40 mL) was added dropwise during 30 min to Mg turnings (2.40 g, 0.1 mol) and ether (300 mL) in a 500 mL flame-dried 3-necked flask equipped with a dry-ice condenser. The light gray mixture was vigorously stirred for 2 h, cooled to -60 °C, and treated with SO₂ for 10 min until there was no further change in appearance of the mixture. The stirred mixture was warmed to room temperature overnight, cooled again to -60 °C, and treated dropwise with SOCl₂ (35 g, 0.30 mol) during 1 h. The mixture was warmed to room temperature, stirred for 4 h, filtered, evaporated, refiltered, and distilled (bp 68–72 °C/20 mmHg), giving the thermally unstable title compound (2.61 g, yield 21%) as a yellow liquid after distillation: ¹H NMR (CDCl₃) δ 5.99 (m, 1 H), 5.59 (m, 2 H), 4.09 (d, *J* = 7.2 Hz, 2 H); ¹³C NMR (CDCl₃) δ 126.32, 124.07, 68.29; IR (ν_{max}, neat) 3088 (w), 2979 (w), 1635 (w), 1338 (m), 1231 (m), 1146 (s), 988 (m), 938 (m) cm⁻¹.

1-Propanesulfinothioic Acid S-Allyl Ester (10). A solution of 1-propanesulfinyl chloride (1.9 g, 0.015 mol) (Block *et al.*, 1996c) in ether (30 mL) was added dropwise to a mixture of 2-propenethiol (1.1 g, 0.015 mol), pyridine (3 mL), and ether (30 mL) at 0 °C in a flame-dried 3-necked 100 mL flask. The cold solution, now containing a heavy white precipitate, was stirred for 1 h and then extracted with chilled H₂SO₄ (1 M, 3 × 20 mL). The ether layer was separated, and the combined aqueous layers were saturated with ammonium sulfate and extracted with CH₂Cl₂ (3 × 20 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo affording thermally unstable 10 as a colorless liquid with a garlic-like odor (2.04 g, 83% yield): ¹H NMR (CDCl₃) δ 5.88 (m, 1 H), 5.21 (m, 2 H), 3.73 (m, 2 H), 3.07 (m, 2 H), 1.83 (sextet, *J* = 7.8 Hz, 2 H), 1.03 (t, *J* = 7.2 Hz, 3 H); ¹³C NMR (CDCl₃) δ 132.96, 118.93, 57.76, 35.35, 17.38, 13.37; IR (ν_{max}, neat) 3083 (w), 2965 (s), 2931 (m), 1635 (w), 1083 (s), 987 (m), 918 (s) cm⁻¹.

2-Propene-1-sulfinothioic Acid S-Propyl Ester (11). Reaction of 2-propenesulfinyl chloride (1.24 g, 0.01 mol) and 1-propanethiol (0.76 g, 0.01 mol) in ether in the presence of pyridine followed by workup as above gave thermally unstable 11 (1.46 g, yield 89%) as a slightly yellow liquid with a garlic-like odor: ¹H NMR (CDCl₃) δ 5.91 (m, 1 H), 5.42 (m, 2 H), 3.77 (oct., *J* = 7.8 Hz, 2 H), 3.10 (m, 2 H), 1.80 (sextet, *J* = 7.2 Hz, 2 H), 1.02 (t, *J* = 7.2 Hz, 3 H); ¹³C NMR (CDCl₃) δ 125.93, 123.86, 60.26, 36.27, 24.14, 13.38; IR (ν_{max}, neat) 3084 (w), 2963 (s), 2928 (m), 1635 (m), 1457 (m), 1378 (m), 1292 (m), 1093 (s), 988 (w), 928 (m) cm⁻¹.

RESULTS AND DISCUSSION

Garlic and Ramp. Alliin (9) and related thiosulfonates, [RS(O)SR', 1–5, 8, 10–14; see Tables 2 and 3 for compound identification] were identified in SF extracts of fresh garlic and/or frozen and fresh ramp homogenates. For the purposes of the discussion below allyl (All) refers to the 2-propenyl substituent. The LC-APCI-MS total ion chromatograms (TICs) of garlic and ramp SF extracts are shown in Figure 1. Under APCI conditions with an acetonitrile/water LC mobile phase, the mass spectra of the thiosulfonates have a base peak corresponding to the protonated molecule (MH⁺). An acetonitrile adduct ion (M + CH₃CN)⁺ was also observed. Capillary temperature and/or auxiliary gas flow rate can be adjusted to minimize the formation of the adduct ion. Fragment ions become prominent as the capillary temperature is increased and/or the auxiliary gas flow is decreased. A major fragment ion that is present for alliin (9) and its allyl 1-propenyl isomers (12–14), under certain conditions, was *m/z* 121 which is consistent with the loss of the allyl functionality, *m/z* 41.

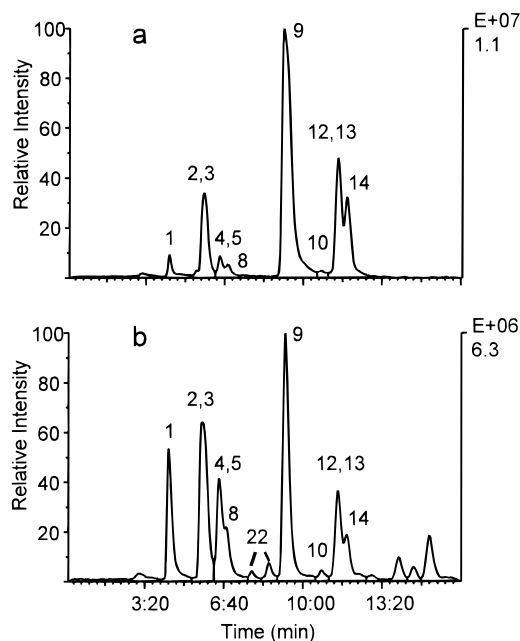


Figure 1. Total ion chromatograms representing typical SF extracts of (a) garlic and (b) ramp. See Table 2 for compound identification.

Table 1. Percent Peak Areas^a Corresponding to the Various Thiosulfinates in the Garlic and Ramp Extracts

compd no.	compd ^b	fresh garlic	frozen ramp ^c
1	MeS(O)SMe	1.8 (0.9)	15.1
2, 3	MeS(O)SAl/MeSS(O)AlI	18.0 (18.5)	17.2
4, 5, 8	MeS(O)S1-propenyl-(<i>E,Z</i>)/MeSS(O)1-propenyl-(<i>E</i>)	7.2 (9.4)	25.3
9	AllS(O)SAlI	53.4 (59)	26.7
10	<i>n</i> -PrS(O)SAlI	<i>d</i>	1.2
12, 13, 14	AllS(O)S1-propenyl-(<i>E,Z</i>)/AllSS(O)1-propenyl-(<i>E</i>)	19.7 (19.6)	14.5

^a The values of the peak areas are calculated from the TICs. Values in parentheses are calculated mol % as reported by Block *et al.* (1992a). Extracts were analyzed within 1–6 h of extraction and kept on dry ice until analyzed. ^b See Table 2 for *Chemical Abstracts* names of compounds. ^c Fresh ramp bulbs were frozen at -80°C until extracted. ^d The response for this compound was observed in the selected ion trace (m/z 165) but not integrated in the TIC.

The relative amounts of various thiosulfinates, based on percent peak areas from the TIC, were different in the garlic and ramp extracts (Table 1). The major thiosulfinate in garlic extracts was allicin (**9**, $\approx 53\%$ of calculated peak areas for total thiosulfinates) with significant contributions from the mixed allyl methyl thiosulfinates (**2, 3**, $\approx 18\%$), the mixed methyl 1-propenyl thiosulfinates (**4, 5, 8**, $\approx 7\%$) and the mixed allyl 1-propenyl thiosulfinates (**12–14**, $\approx 19\%$). In the frozen ramp extract the relative abundance (RA) of allicin (**9**, $\approx 26\%$) was less than that found in typical garlic extracts, while the RAs of dimethyl thiosulfinate (**1**, $\approx 15\%$) and mixed methyl 1-propenyl thiosulfinates (**4, 5, 8**, $\approx 25\%$) were significantly higher. Botanically, ramp is closely related to European and Asian varieties of wild garlic (*A. ursinum* and *A. victorialis*; personal communication, Dr. Andrée Nault, 1996). The amount of allicin (**9**) relative to the other thiosulfinates found in the frozen ramp extract was similar to those reported for wild garlic although the 1-propenyl functionality was more abundant in the former than in the latter (Block *et al.*, 1992a).

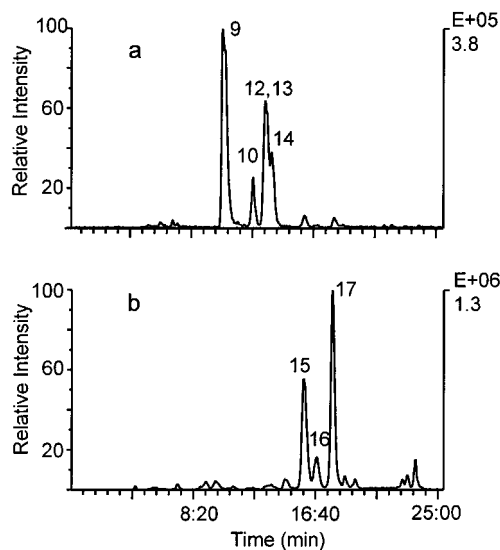


Figure 2. Selected ion chromatograms (m/z 165) representing typical SF extracts of (a) ramp (garlic afforded a similar chromatogram for compounds **9**, **10**, and **12–14**) and (b) yellow onion. See Table 2 for compound identification.

Tables 2 and 3 list the MS-MS spectral data for the MH^+ ions of thiosulfinates and related compounds found in extracts of *Allium* species. The fragmentation pattern of the MS-MS spectra of the C_4 thiosulfinates, the methyl allyl thiosulfinates (**2, 3**) and the methyl 1-propenyl thiosulfinates (**4, 5, 8**), permitted identification of these isomers. The base peak for the isomers **4, 5**, and **8** was the protonated parent (m/z 137) while the base peaks for the isomers **2** and **3** were fragment ions at m/z 73 and 41, respectively. In all cases at least one unique ion was present in each isomer group to distinguish the regioisomers [*i.e.*, $\text{RS}(\text{O})\text{SR}'$ vs $\text{RSS}(\text{O})\text{R}'$]. Likewise, the MS-MS spectra of the C_6 thiosulfinates, allicin (**9**) and its allyl 1-propenyl isomers (**12–14**), were sufficiently distinctive to permit assignment of the chromatographic peaks. Consequently, we determined that the chromatographic separation of the thiosulfinates **12–14** reversed order when acetonitrile/water was used as the mobile phase instead of methanol/water (Calvey *et al.*, 1994).

The LC-MS data provided convincing evidence that thiosulfinates containing the *n*-propyl moiety were present in garlic and ramp SF extracts. Boscher *et al.* (1995) previously reported the presence of trace amounts of *n*-propyl-containing sulfides and polysulfides in garlic extracts or distilled garlic oils. Edwards *et al.* (1994) reported the presence of *S-n*-propyl L-cysteine *S*-oxide in trace amounts in garlic. The latter compound would be the precursor of thiosulfinates containing the *n*-propyl moiety in garlic extracts. A peak (**10**, $t_R \approx 12.5$ min) eluting between allicin and thiosulfinates **12–14** was observed in the SF extracts of garlic and ramp homogenates (Figure 2a) with an apparent MH^+ ion at m/z 165 and a corresponding acetonitrile adduct ion at m/z 206. This peak contrasted with the elution of 1-propenyl *n*-propyl isomers (**15–17**, MH^+ , m/z 165, t_R between 15 to 18 min; Figure 2b; also compare m/z 163 and 165 traces in Figure 6) observed in onion juice extracts, which eluted after the isomers **12–14**. Authentic standards were synthesized to confirm the presence of these *n*-propyl-containing thiosulfinates in fresh garlic and ramp extracts. The previously unknown isomers 1-propanesulfinothioic acid *S*-allyl ester [**10**, *n*-PrS(O)SCH₂CH=CH₂] and 2-propene-1-sulfinothioic acid *S*-propyl ester [**11**, *n*-PrSS(O)CH₂CH=CH₂]

Table 2. APCI-MS-MS Data of Thiosulfonates Found in Extracts of *Allium* spp.

cpd no.	compound ^a	MS-MS spectral data of MH ⁺ ions (relative abundance)
1	MeS(O)SMe	111 (100); 65 (68); 63 (56); 49 (24)
2	MeS(O)SAll	137 (60); 135 (25); 73 (100); 64 (10); 47 (8); 45 (45); 41 (58); 39 (20)
3	MeSS(O)All	137 (45); 135 (10); 95 (24); 79 (22); 64 (5); 47 (5); 45 (6); 41 (100); 39 (8)
4, 5	MeS(O)S1-propenyl-(<i>E,Z</i>)	137 (100); 136 (22); 120 (6); 73 (74); 64 (47); 47 (14); 45 (66); 41 (40); 39 (20); 29 (12)
6	MeSS(O)Pr- <i>n</i>	139 (100); 97 (30); 79 (74); 73 (12); 59 (16); 43 (70)
7	MeS(O)SPr- <i>n</i>	139 (100); 97 (64); 75 (44); 43 (48)
8	MeSS(O)1-propenyl-(<i>E</i>)	137 (100); 136 (35); 120 (4); 90 (44); 64 (6); 47 (8); 45 (36); 41 (30); 39 (18); 29 (8)
9	AllS(O)SAll	163 (7); 121 (12); 105 (4); 93 (3); 87 (10); 73 (100); 41 (29)
10	<i>n</i> -PrS(O)SAll	165 (14); 73 (100)
11	<i>n</i> -PrSS(O)All	165 (14); 123 (90); 107 (10); 89 (14); 73 (30); 43 (100); 41 (16)
12, 13	AllS(O)S1-propenyl-(<i>E,Z</i>)	163 (10); 121 (65); 105 (30); 103 (18); 93 (19); 87 (100); 81 (28); 73 (10); 59 (20); 55 (8); 41 (18)
14	AllSS(O)1-propenyl-(<i>E</i>)	163 (10); 121 (56); 105 (18); 103 (15); 93 (16); 87 (71); 81 (15); 73 (100); 59 (10); 55 (8); 41 (14)
15	<i>n</i> -PrS(O)S1-propenyl-(<i>E</i>) ^b	165 (72); 105 (34); 73 (100); 43 (24)
16	<i>n</i> -PrS(O)S1-propenyl-(<i>Z</i>) ^b	165 (40); 105 (64); 73 (100); 43 (20)
17	<i>n</i> -PrSS(O)1-propenyl-(<i>E</i>) ^b	165 (42); 105 (2); 89 (100); 61 (8); 43 (26)
18	<i>n</i> -PrS(O)SPr- <i>n</i>	167 (100); 125 (32); 107 (63); 93 (11); 75 (16); 73 (74); 59 (10); 43 (42)

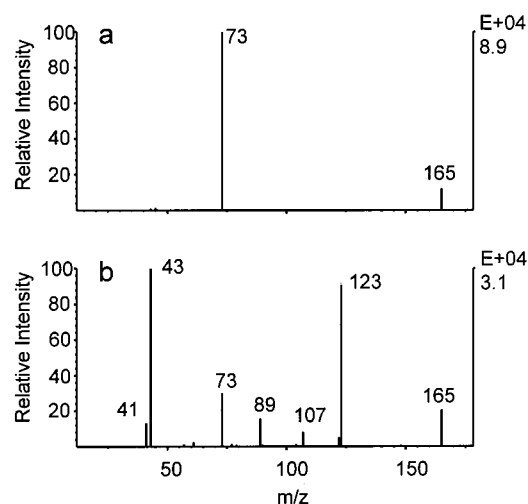
^a Chemical Abstracts names for compounds: **1**, methanesulfinothioic acid *S*-methyl ester; **2**, methanesulfinothioic acid *S*-2-propenyl ester; **3**, 2-propene-1-sulfinothioic acid *S*-methyl ester; **4**, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **5**, methanesulfinothioic acid *S*-(*Z*)-1-propenyl ester; **6**, 1-propanesulfinothioic acid *S*-methyl ester; **7**, methanesulfinothioic acid *S*-*n*-propyl ester; **8**, (*E*)-1-propenesulfinothioic acid *S*-methyl ester; **9**, 2-propene-1-sulfinothioic acid *S*-2-propenyl ester (allicin); **10**, 1-propanesulfinothioic acid *S*-2-propenyl ester; **11**, 2-propene-1-sulfinothioic acid *S*-*n*-propyl ester; **12**, 2-propene-1-sulfinothioic acid *S*-(*E*)-1-propenyl ester; **13**, 2-propene-1-sulfinothioic acid *S*-(*Z*)-1-propenyl ester; **14**, (*E*)-1-propenesulfinothioic acid *S*-2-propenyl ester; **15**, 1-propanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **16**, propanesulfinothioic acid *S*-(*Z*)-1-propenyl ester; **17**, (*E*)-1-propenesulfinothioic acid *S*-*n*-propyl ester; **18**, propanesulfinothioic acid *S*-*n*-propyl ester. ^b Elution order based on reversal of Si-HPLC data reported by Block *et al.* (1992).

Table 3. APCI-MS-MS Data of Bissulfine, Zwiebelanes, Cepaenes, and Ajoene Found in Extracts of *Allium* spp.

cpd no.	compound ^a	MS-MS spectral data of MH ⁺ ions (relative abundance)
19	<i>d,l</i> -OSCH(CHMe) ₂ CHSO	179 (100); 161 (15); 133 (35); 127 (6); 115 (35); 113 (36); 99 (12); 97 (10); 87 (6); 69 (22); 43 (6)
20	<i>cis</i> -zwiebelane	163 (24); 113 (100); 101 (4)
21	<i>trans</i> -zwiebelane	163 (46); 113 (100); 101 (12)
22	MeS(O)CHEtSSMe ^b	185 (30); 121 (70); 111 (10); 79 (98); 73 (100); 64 (16); 45 (22)
23	1-propenylS(O)CHEtSSMe ^c	211 (12); 121 (65); 79 (98); 73 (100); 64 (12); 45 (24); 41 (10)
24	MeS(O)CHEtSS1-propenyl ^d	211 (35); 147 (75); 105 (100); 73 (70); 61 (34); 45 (24); 41 (25)
25	1-propenylS(O)CHEtSS1-propenyl ^e	237 (10); 147 (50); 105 (100); 73 (45); 61 (30); 45 (24); 41 (10)
26	1-propenylS(O)CHEtSSPr- <i>n</i> ^f	239 (6); 149 (48); 107 (20); 73 (100); 45 (16); 43 (40); 41 (10)
27	<i>n</i> -PrS(O)CHEtSS1-propenyl ^d	239 (4); 147 (100); 105 (76); 73 (30); 61 (20); 45 (12); 41 (20)
28	AllS(O)CH ₂ CH=CHSSAll ^g	235 (16); 145 (19); 111 (18); 103 (100); 73 (92); 67 (28); 45 (35); 41 (24)

^a Chemical Abstracts names for the compounds: **19**, (*Z,Z*)-*d,l*-2,3-dimethyl-1,4-butanedithial *S,S*-dioxide (bissulfine); **20** (1 α ,2 α ,3 α ,4 α ,5 β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide (*cis*-zwiebelane); **21**, (\pm)-(1 α ,2 α ,3 β ,4 α ,5 β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide (*trans*-zwiebelane); **22**, methyl 1-(methylsulfinyl)propyl disulfide; **23**, methyl (*E*)-1-(1-propenylsulfinyl)propyl disulfide; **24**, 1-(methylsulfinyl)propyl (*E,Z*)-1-propenyl disulfide; **25**, (*E*)-1-propenyl 1-(1-propenylsulfinyl)propyl disulfide; **26**, (*E*)-1-(1-propenylsulfinyl)propyl propyl disulfide; **27**, (*E*)-1-propenyl 1-(propylsulfinyl)propyl disulfide; **28**: (*E,Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide ((*E,Z*)-ajoene). ^b Observed two chromatographic peaks. Both chromatographic peaks resulted in similar product ion mass spectra. These were assumed to be chromatographically resolved diastereomers due to chirality at both sulfur and carbon. ^c All synthesized isomers produced similar spectra. Only observed one chromatographic peak for the *E* configuration eluting at \approx 16 min. The diastereomers with the *Z* configuration eluted between 13 and 14 min; these two peaks were baseline resolved. ^d Product ion spectra of the mixed cepaenes found in the onion extracts. Assignments were made based on synthetic standards. ^e Observed only one chromatographic peak, indicating that diastereomers may not be resolved. ^f All synthesized isomers produced similar spectra. Observed two chromatographic peaks for the *Z* configuration eluting between 23 and 24 min; these peaks were not baseline resolved. Only one peak was observed for the *E* configuration eluting \approx 22.5 min. ^g Only observed in garlic extracts; eluting at \approx 19 min.

were prepared by a pyridine-catalyzed condensation of the corresponding sulfinyl chlorides and thiols. 2-Propenesulfinyl chloride, which has not been previously reported, was prepared by treatment of the magnesium salt of 2-propenesulfinic acid with thionyl chloride. Compounds **10** and **11** were well separated by reversed-phase LC and showed distinctly different fragmentation patterns with APCI-MS-MS analysis. The APCI-MS-MS (Figure 3) data confirmed that compound **10** was present in extracts of ramp and garlic homogenates. The data suggested that compound **11** was present, although it was not resolved from the allyl 1-propenyl thiosulfonates (**12**–**14**). Extracts of fresh garlic homogenates obtained at various times of the year have consistently shown evidence of trace levels of **10**. The relative amount of **10** based on peak area is \approx 0.5% of allicin. *n*-Propyl-containing thiosulfonates were previously thought to be absent in garlic (Koch and Lawson, 1996; Lawson *et al.*, 1991; Block, 1992a).

**Figure 3.** APCI-MS-MS spectra of (a) compound **10** [*n*-PrS(O)SAll] and (b) compound **11** [*n*-PrSS(O)All].

The methyl *n*-propyl thiosulfates (**6**, **7**, MH⁺, *m/z* 139), minor components in the SF extracts of ramp, coelute with the methyl 1-propenyl isomers (**4**, **5**, **8**, MH⁺, *m/z* 137). Their presence was suggested because the abundance of the ion at *m/z* 139 (≈20% RA) relative to the ion at *m/z* 137 can not be fully explained as the isotope ion (9.2% calculated RA) associated with **4**, **5**, and **8** (MH⁺, *m/z* 137).

The data for a late eluting constituent (*t_R* ≈ 19–20 min) in garlic extracts (not shown) were consistent with data for ajoene [**28**, ALLS(O)CH₂=CHSSAll], a major component found in oil-macerated garlic products (Lawson, 1993; Block *et al.*, 1986). Although ajoene was not conclusively identified in ramp extracts, there is some evidence that ajoene-related compounds, where methyl functionalities are substituted for the allyl groups [MeS(O)CH₂CH=CHSSMe, (*E,Z*)-2,3,7-trithiaoct-4-ene 7-oxide, **29**], are present in SF extracts of ramp homogenates. Sendl *et al.* (1991, 1992a,b) previously isolated ajoene homologs, **29** and (*E,Z*)-4,5,9-trithiaocta-1,6-diene 9-oxide [MeS(O)CH₂CH=CHSSCH₂CH=CH₂, **30**] from chloroform and chloroform/acetone extracts of wild garlic. Sendl also reported that these compounds were found in garlic extracts but ajoene predominated. Ajoene is structurally related to the cepaenes [RS(O)CH₂EtSSR'] found in onion extracts. Ramp extracts also appear to contain cepaenes, some of which have MH⁺ ions and retention times consistent with the methyl 1-(methylsulfinyl)propyl disulfide (**22**), methyl (*E,Z*)-1-(1-propenylsulfinyl)propyl disulfide (**23**), and 1-(methylsulfinyl)propyl (*E,Z*)-1-propenyl disulfide (**24**) isomers. Other components, which may be particular cepaenes, had retention times that differed from those observed for the cepaenes found in onion extracts or for the synthetic standards analyzed. Their chromatographic retention times suggested the incorporation of the allyl group into the cepaene backbone (Calvey *et al.*, 1996). Morimitsu *et al.* (1992) reported the formation of allyl containing cepaenes in extracts of homogenates prepared from combined onion and garlic. Other late eluting constituents observed in garlic and ramp extracts by LC with UV detection were not readily observed by LC–MS in the TIC. These components have retention times and UV spectra characteristic of the dithiins, alkyl sulfides, and polysulfides (Calvey and Block, 1997), which ionize poorly under the APCI conditions employed in this study.

Onion. The major sulfur constituents in an SF extract of onion, as determined by cryogenic GC–MS, are methanesulfinothioic acid *S*-(*E,Z*)-1-propenyl ester (**4,5**), (*E*)-1-propenesulfinothioic acid *S*-methyl ester (**8**), (1 α ,2 α ,3 α ,4 α ,5 β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide (*cis*-zwibelane, **20**), (\pm)-(1 α ,2 α ,3 β ,4 α ,5 β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide (*trans*-zwibelane, **21**), and (*E,Z*)-propanethial *S*-oxide (lachrymatory factor, LF) (Calvey *et al.*, 1994). SF extracts rapidly prepared from the juice of 10–15 g of fresh yellow onions and immediately analyzed by LC–MS showed the presence of various thiosulfates, a bisulfine, zwibelanes, and significant quantities of a series of cepaenes. Identification of the organosulfur compounds was achieved by comparison with synthetic standards and further verified by employing LC–MS–MS analyses (Tables 2 and 3). Because of the complexity of the onion extracts, the data obtained for each class of compounds is discussed separately.

(A) *LF*. In onion, the majority of the 1-propenyl group generated as 1-propenesulfenic acid forms the lachry-

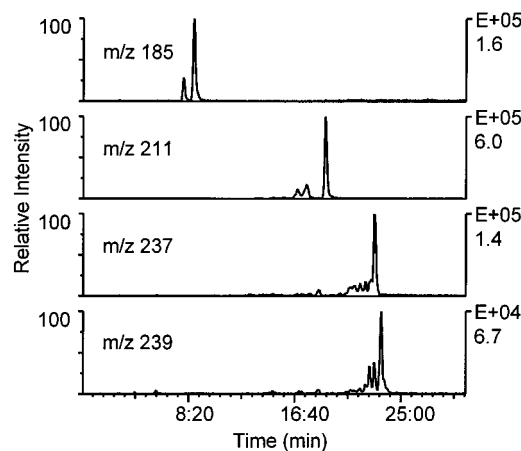


Figure 4. Selected ion chromatograms, from SF extract of fresh onion, for protonated cepaenes **22** (*m/z* 185), **23**, **24** (*m/z* 211), **25** (*m/z* 237), and **26**, **27** (*m/z* 239). See Table 3 for compound identification.

matory factor (Block *et al.*, 1996c). The LF, a strong UV absorber at 254 nm, elutes between compound **1** and bisulfine (**19**). In our mass spectral analyses we generally scanned between 110–350 daltons. Therefore, we did not observe the LF (MH⁺, *m/z* 91) under these conditions. The UV spectrum obtained from LC runs employing a diode array detector indicated its presence in the SF extracts of onion. LC–MS data were then collected by scanning a small mass window from 85 to 98 daltons and the presence of LF in SF extracts of onion was indicated. The low levels of LF were probably due to its extreme volatility and inefficient trapping on glass beads at 0–1 °C (Calvey *et al.*, 1994). More efficient methods for extraction and GC identification of the LF have recently been reported (Schmidt *et al.*, 1996).

(B) *Bisulfine* (**19**). (*Z,Z*)-*d,l*-2,3-Dimethyl-1,4-butane-dithial *S,S*-dioxide has previously been isolated from organic extracts of onion and identified through spectroscopic techniques and synthesis (Block *et al.*, 1996a; Block and Bayer, 1990). Quantitation of the bisulfine in methylene chloride extracts of onion has been achieved by using normal phase LC with UV detection (Block *et al.*, 1992a). The bisulfine was observed in SF extracts of onion via LC–APCI–MS. Under the chromatographic conditions employed, bisulfine eluted before compounds **4** and **5**. The mass spectrum of the bisulfine shows an MH⁺ ion as the base ion at *m/z* 179, two adduct ions at *m/z* 196 (M + H₂O)⁺ and *m/z* 220 (M + CH₃CN)⁺, and fragment ions at *m/z* 161, 133, and 113. MS–MS experiments confirmed that these fragment ions result from decomposition of the MH⁺ ion.

(C) *Cepaenes* (**22–27**). Our LC–MS data indicate that cepaene extraction/formation in SC–CO₂ at 35 °C is rapid and that a series of cepaenes [RS(O)CH₂EtSSR'] are present in an SF extract of onion juice. Each cepaene is a mixture of two diastereomeric pairs due to chirality at both sulfur and carbon. Figure 4 shows detection of a series of diastereomeric cepaenes with MH⁺ ions at *m/z* 185, 211, 237, and 239 [R = R' = Me; R/R' = MeCH=CH, R'/R = Me; R = R' = MeCH=CH; R/R' = MeCH=CH, R'/R = *n*-Pr; each MeCH=CH group is (*E*)- or (*Z*)-]. This was further verified by employing LC–APCI–MS–MS analyses and analyzing synthetic standards (Table 3) (Block *et al.*, 1997). Previously, several laboratories (Kawakishi and Morimitsu, 1988; Bayer *et al.*, 1989) isolated milligram quantities of cepaenes after subjecting kilogram quantities of onion

to organic solvent extraction. After concentration of the extracts, extensive cleanup was achieved through repetitive chromatographic separations. Each group reported the isolation of different cepaenes. Although Breu and Dorsch (1994) reported that cepaenes were the dominant organosulfur compounds found in SF-CO₂ extracts of onion, they did not indicate the SFE conditions employed. Under our SFE conditions the methyl 1-propenyl thiosulfonates and the zwiebelanes generally predominated. Cepaenes are important both in terms of their flavor and biological activity, e.g. as antithrombotic agents (Kawakishi and Morimitsu, 1988; Bayer *et al.*, 1989; Block and Zhao, 1992). The presence of cepaenes and related nonvolatile organosulfur compounds could account for the apparent low SFE efficiency of the thiosulfonates and zwiebelanes previously determined by GC-MS (Calvey *et al.*, 1994).

The "symmetrical" cepaenes MeS(O)CH₂SSMe (**22**, MH⁺, *m/z* 185) and 1-propenylS(O)CH₂SS1-propenyl (**25**, MH⁺, *m/z* 237; geometric isomers) were identified in the onion extracts and verified with authentic standards by using MS-MS. The "mixed" cepaenes, MeS(O)CH₂SS1-propenyl (**24**, MH⁺, *m/z* 211) and *n*-PrS(O)CH₂SS1-propenyl (**27**, MH⁺, *m/z* 239; geometric isomers) found in the onion SF extracts are significantly different from synthetic standards (*E,Z*-1-propenylS(O)CH₂SSMe (**23**, MH⁺, *m/z* 211) and (*E,Z*-1-propenylS(O)CH₂SSPr (**26**, MH⁺, *m/z* 239). Synthetic isomers **23** and **26** eluted prior to 17 min while extract isomers **24** and **27** eluted from 17–23 min. Other differences occurred in the MS-MS spectrum of the MH⁺ ion. Synthetic standards **23** yielded significant product ions at *m/z* 211, 121, 79, and 73. In the onion SF extracts the major chromatographic peak with an MH⁺ ion at *m/z* 211 (*t_R* ≈ 19 min, Figure 4) was tentatively identified as **24** because of the lack of ions at *m/z* 121 and 79 and the presence of ions at *m/z* 147 and 105 in the MS-MS spectrum. The component (MH⁺, *m/z* 211) in the onion extract eluting between 16–17 min was tentatively identified as (*E*)-**23** due to the similar MS-MS spectrum of the synthetic standard. The component (MH⁺, *m/z* 211) with *t_R* ≈ 17.5 min has an MS-MS spectrum similar to the major *m/z* 211 component, **24**. Similarly, the major chromatographic peak with an MH⁺ ion at *m/z* 239 (*t_R* ≈ 23 min, Figure 4) was tentatively identified as **27** because of the presence of ions at *m/z* 147 and 105 and the lack of ions at *m/z* 149 and 107 in the MS-MS spectrum. The ions at *m/z* 147 and 105, observed in the MS-MS spectrum of the "symmetrical" cepaenes containing the 1-propenyl group, can be attributed to the ions MeCH=CHS₂⁺ and MeCH=CHS₂CH₂E⁺, respectively. These tentative identifications were later verified with synthetic standards of **24** and **27**.

(D) *Zwiebelanes* (**20**, **21**). Previous data obtained by the authors have shown that the zwiebelanes are major sulfur containing compounds found in onion extracts (Block *et al.*, 1992b; Calvey *et al.*, 1994). Using a C₁₈ column and a CH₃CN/H₂O gradient, the zwiebelanes (**20**, **21**) were not baseline resolved from the methyl cepaenes (**22**). They can be resolved by mass spectrometry, however, on the basis of selected ion traces of the MH⁺ ions, *m/z* 163 and 185, respectively (Figure 5). The MS-MS spectra of the MH⁺ ions of these two zwiebelanes showed a base peak at *m/z* 113. The spectra differ in the intensity of the parent ion (MH⁺, *m/z* 163). The *trans* isomer (**21**) has approximately double the intensity of the *cis* isomer (**20**). The *trans* isomer (**21**)

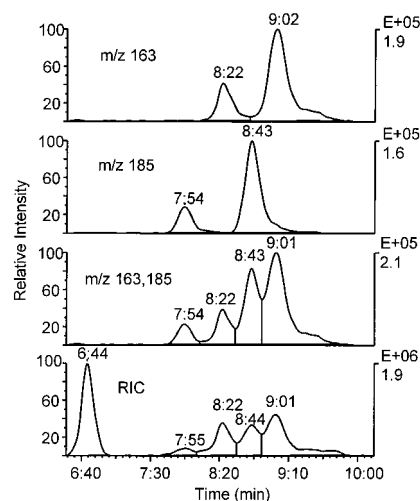


Figure 5. Selected ion chromatogram from SF extract of fresh onion showing spectrometric resolution of the protonated zwiebelanes **20**, **21** (*m/z* 163) and "methyl/methyl cepaenes" **22** (*m/z* 185). See Table 3 for compound identification.

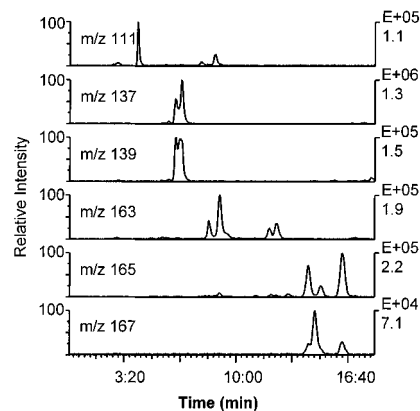


Figure 6. Selected ion chromatograms of protonated thiosulfonates found in onion extracts: **1** (*m/z* 111), **4**, **5** (*m/z* 137), **6**, **7** (*m/z* 139), **12**–**14** (*m/z* 163; also shows zwiebelanes **20**, **21** as 8–9 min retention time peaks), **15**–**17** (*m/z* 165), and **18** (*m/z* 167). See Tables 2 and 3 for compound identification.

was the major component of the two and eluted after the *cis* isomer (**20**). There also appeared to be a coeluting compound in the onion extract that produced an isobaric ion at *m/z* 163. This is evidenced by a significant product ion at *m/z* 73, which is not observed in appreciable amounts in the spectra of the zwiebelane synthetic standards. This may suggest the presence of alliin (**9**), but due to its low concentration in the extracts, conclusive MS-MS evidence was not obtainable.

(E) *Thiosulfonates*. A series of thiosulfonates was found in the onion SF extract (Figure 6). We have previously quantitated some of these compounds by GC-MS in a single SF extract of onion (Calvey *et al.*, 1994). The methanesulfinothioic acid *S*-methyl ester (**1**) and propanesulfinothioic acid *S*-*n*-propyl ester (**18**) were not determined by GC-MS because different chromatographic conditions were required to determine their presence in onion extracts. The major thiosulfonates found in multiple SF extracts of onion were the methyl 1-propenyl isomers (**4**, **5**, **8**, MH⁺, *m/z* 137). Under the LC conditions employed in this study, the isomers were not baseline-resolved. The methyl *n*-propyl isomers (**6**, **7**, MH⁺, *m/z* 139) are minor components in the SF extracts of onion and these coeluted with **4**, **5**, and **8**. Their presence was suggested because the RA of the ion at *m/z* 139 (11–20%) relative to the ion at *m/z* 137

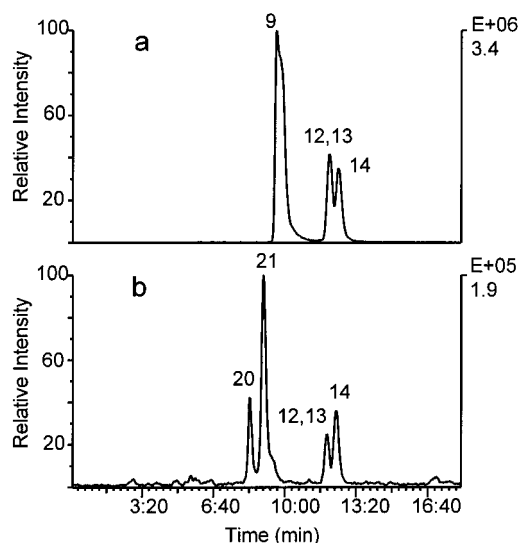


Figure 7. Selected ion chromatogram (m/z 163) from SF extract of (a) fresh garlic homogenate and (b) fresh onion juice suggesting the presence of the allylic compounds **12–14** in onion extracts. See Table 2 for compound identification.

cannot be fully explained as the isotope ion (9.2% calculated RA) associated with the methyl 1-propenyl components (MH^+ , m/z 137). Three 1-propenyl *n*-propyl isomers (**15–17**, MH^+ , m/z 165) are found in fresh onion extracts with retention times between 14 and 16.5 min. Propanesulfinothioic acid *S-n*-propyl ester (**18**, MH^+ , m/z 167) is observed in SF extracts of onion eluting between the *n*-PrS(O)S1-propenyl-(*E,Z*) isomers (**15**, **16**, MH^+ , m/z 165).

Review of the LC–MS data of three extracts from two different onions showed retention times similar to garlic extracts for ions of allyl 1-propenyl thiosulfates (**12–14**) that indicate their presence in smaller quantities in onion extracts (Figure 7). The ion at m/z 121, observed in the mass spectra, provides additional support for their presence in the onion extracts (see discussion on garlic and ramp). The relative amounts of **12–14** based on the combined peak areas for **4**, **5**, and **8** from the TIC is $\approx 7\%$. As we have previously reported, the concentration of the thiosulfates in onion juice is ≈ 100 -fold less than that found in extracts of garlic homogenates (Block *et al.*, 1992a; Calvey *et al.*, 1994). The low concentration of **12–14** in the extracts makes it difficult to obtain conclusive MS–MS evidence of their presence. The occurrence of these compounds could not be determined by GC–MS because of their thermal instability. Traces of precursor (+)-*S*-allyl L-cysteine *S*-oxide were found in onion (Edwards *et al.*, 1994). Furthermore, GC–MS analysis established the presence of low levels of polysulfides containing the allyl group in distilled onion oil (Brodnitz *et al.*, 1968; Boelens *et al.*, 1971; Kallio and Salarinne, 1990; Tokitomo and Kobayashi, 1992).

CONCLUSION

LC–APCI–MS methods provide an effective alternative for analysis of thermally labile organosulfur compounds found in extracts of *Allium* spp. By employing LC–MS we confirmed the presence of *n*-propyl-containing thiosulfates in extracts of garlic and ramp homogenates. The low UV absorbance, low concentration, and thermal lability of these compounds made detection by other means difficult. While APCI–MS may give ambiguous data based solely on the protonated molecule and adduct ions, the MS–MS spectra show that the

regioisomers of the various sulfur-containing compounds present can be uniquely distinguished. The MS conditions employed in this study are sufficiently energetic to ionize sulfinyl [$-S(O)-$] compounds. Sulfides and related compounds involving divalent sulfur were not observed, although their presence was suggested by LC–UV analysis.

ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; GC, gas chromatography; GC–MS, coupled gas chromatography–mass spectrometry; LC, liquid chromatography; LC–APCI–MS, coupled liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry; LC–MS, coupled liquid chromatography–mass spectrometry; MH^+ , protonated molecule; MS, mass spectrometry; MS–MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; RA, relative abundance; t_R , retention time; SC-CO₂, supercritical carbon dioxide; SF, supercritical fluid; SFC, supercritical fluid chromatography; SFC–MS, coupled supercritical fluid chromatography–mass spectrometry; SFE, supercritical fluid extraction; TIC, total ion chromatogram.

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