# Characterization of Silylated Desulfoglucosinolate Mixtures Using High-Performance Capillary Gas Chromatography (GC)–Negative-Ion Chemical Ionization Mass Spectrometry (NICIMS) and GC–NICIMS/MS

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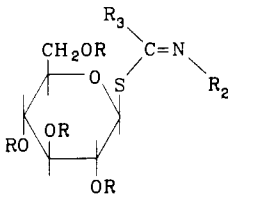
Glucosinolates are important constituents of vegetables of the *Brassica* family because of their physiological effects in various animals and recent studies that suggest that in some cases glucosinolates confer resistance to chemical carcinogenesis. Although both the positive-ion (PI) electron ionization and chemical ionization mass spectra of various glucosinolates and desulfoglucosinolates have been reported, there are very few mass spectral data available on the per(trimethylsilyl)desulfo analogues. This paper describes the characterization of mixtures of both standard and naturally occurring glucosinolates as their per(trimethylsilyl)desulfoglucosinolate analogues by capillary gas chromatography (GC)–negative-ion chemical ionization mass spectrometry (NICIMS) and GC–NICIMS/MS. Sensitivity measurements showed that NICI gave an approximately 10-fold improvement in glucosinolate detection, compared to PICI. NICI mass spectra revealed that a significant proportion of the total ion current for each compound was accounted for by four diagnostic ions. This information was used for rapid class identification from reconstructed total ion current chromatograms.

Glucosinolates (1) are ubiquitous among cruciferous plants and vegetables. This class of β-D-thioglucosides is particularly important because of recent evidence that certain of these compounds, present in the genus *Brassica*, may act as inhibitors of carcinogenesis (National Research Council, 1982). Further, the associated end products formed from the endogenous enzyme hydrolysis of glucosinolates in disrupted plant tissue carry a wide spectrum of biological activities which include both beneficial and adverse physiological effects. Fenwick et al. (1983) have presented a historical perspective and discussion of the physiological effects of glucosinolates and their breakdown products in foods.

In the family Cruciferae, which includes such plants as cabbage, cauliflower, Brussels sprouts, kale, and rape, the presence or absence of specific glucosinolates has important economic implications. Accordingly, a number of analytical techniques (McGregor et al., 1983) have been developed to identify and quantitate glucosinolates either collectively or specifically in plant tissues and associated processed products.

The recent trend in the analysis of glucosinolates has been toward more direct rapid and reliable chromatographically based techniques such as liquid chromatography (Bradfield and Bjeldanes, 1987) and liquid chromatography–mass spectrometry (Hogge et al., 1987) of desulfoglucosinolates (2) or capillary gas chromatography (GC) (Sosulski and Dabrowski, 1984), gas chromatography–mass spectrometry (GC–MS) (Shaw and Andrzejewski, 1987), GC–MS/MS (Shaw et al., 1989), and GC matrix isolation Fourier transform infrared spectrometry (Mossoba et al., 1989) of the more relatively volatile per(trimethylsilyl)desulfoglucosinolates (3).

The use of GC–MS and GC–MS/MS in conjunction with soft ionization techniques such as positive-ion chemical ionization (PICI) (Christensen et al., 1982; Eagles et al., 1981; Shaw and Andrzejewski, 1987; Shaw et al., 1989) has been shown to offer diagnostically valuable information that, when combined with capillary GC retention time data for individual glucosinolates, leads to unequivocal glucosinolate identification.




| STRUCTURE | R | $R_2$ | $R_3$ |
|---|---|---|---|
| 1 | H | $OSO_3^-$ | side chain |
| 2 | H | OH | side chain |
| 3 | TMS | OTMS | side chain |

As part of our continuing interest in the qualitative analysis of glucosinolates from *Brassica* vegetables, we have recently investigated the utility of GC–MS and GC–MS/MS using negative-ion chemical ionization (NICI) mass spectrometry. We report the characteristics of GC–NICIMS and GC–NICIMS/MS using methane as the reagent gas in the analysis of per(trimethylsilyl)desulfoglucosinolate mixtures isolated from selected vegetables.

## EXPERIMENTAL SECTION

**Materials.** Fresh samples of rutabaga (swede) were purchased from a local wholesale outlet. Methanol was "Baker Analyzed" reagent grade from J. T. Baker Chemical Co. (Phillipsburg, NJ). Single-distilled, deionized water was used for extractions and ion-exchange column chromatography. DEAE Sephadex A-25, SP-Sephadex C-25 resins, and aryl sulfatase (Type H-1) were purchased from Sigma Chemical Co. (St. Louis,

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MO). Pyridine (silylation grade), trimethylchlorosilane (TMCS), and *N*-methyl-*N*-(trimethylsilyl)heptafluorobutyramide (MSHFBA) were purchased from Alltech (Deerfield, IL). Silylation reagents were stored in a dry environment at 4 °C. Glucosinolate standards either were purchased from Atomergic Chemetals Corp. (Farmingdale, NY) or were gifts.

**Instrumentation.** Low-resolution GC–NICIMS and GC–NICIMS/MS analyses were performed on a triple-stage quadrupole Finnigan TSQ46 mass spectrometer (San Jose, CA) equipped with an Incos 2300 data system (including TSQ software, revision C). This instrument was modified with a TSQ offset analyzer board that ramps the offset bias of the third quadrupole as a function of mass to alter transmission of the daughter ions at low mass. High-resolution mass measurements were performed on a VG-ZAB-2F mass spectrometer (Manchester, England) interfaced to an Incos 2300 data system with a Nova 4X computer (revision 6.5 software).

**Sample Preparation.** Vegetable samples were extracted and the glucosinolate fraction was desulfated by using minor modifications (Shaw et al., 1989) to the procedure of Heaney and Fenwick (1980).

**Silylation of Desulfoglucosinolate Mixtures.** Dried extracts containing desulfoglucosinolates were derivatized as previously described (Shaw et al., 1989).

**Capillary GC–NICIMS.** Silylated desulfoglucosinolate mixtures were separated on a fused-silica WCOT capillary column (25 m × 0.2 mm) wall-coated with a 0.33-μm film of chemically bonded methylsilicone (HP-Ultra 1, Hewlett-Packard). GC temperature programming conditions and GC–MS interface conditions have been described (Shaw et al., 1989).

Conventional NICI mass spectra were acquired by configuring the first set of quadrupole rods, $Q_1$, to scan from $m/z$ 60 to $m/z$ 660 in 1.0 s and for $Q_2$ and $Q_3$ to pass all ions. Optimal mass resolution and peak shape were established by using perfluorotributylamine under NICI conditions with methane as the reagent gas (0.25 Torr). The instrument parameters were as follows: electron energy, 70 eV; emission current, 0.4 mA; source temperature, 140 °C; conversion dynode, 5 kV; preamplifier, $10^{-8}$ A/V.

**Capillary GC–NICIMS/MS.** Daughter ion mass spectra were recorded by scanning $Q_3$ (20–350 Da) for anions resulting from the collision-induced dissociation (CID) in $Q_2$ of a precursor ion formed under NICI (methane) ionization conditions in $Q_1$. $Q_2$ was maintained at an argon target gas pressure of 1.8–2.0 mTorr and configured to pass all ions formed through CID. The selected collision energy was +20 eV. Electron energy and scan rate were the same as described in the preceding section.

**High-Resolution Capillary GC–NICIMS.** Capillary column and GC conditions were identical with those described by Shaw et al. (1989). The instrument was operated at 100-eV electron energy, 0.5-mA emission current, 220 °C source temperature, $10^{-7}$ A/V preamplifier, and 10 000 base-line resolution. NICI conditions were obtained with methane as the reagent gas ($2.5 \times 10^{-5}$ Torr). High-resolution data were acquired by using voltage scanning over a narrow range covering the ion of interest.

## RESULTS AND DISCUSSION

**Capillary GC–NICIMS.** In this study, a series of glucosinolate standards and extracts from rutabaga were desulfated and silylated under conditions identical with those reported previously for the routine analysis of glucosinolates in *Brassica* vegetables (Shaw et al., 1989). A representative reconstructed total ion current (TIC) chromatogram from the GC–NICIMS analysis of a rutabaga extract is shown in Figure 1. Despite the complexity of the biological matrix and the continuing difficulties with the chemical instability of 3-(methylsulfinyl)propyl (12D) and (1-methoxy-3-indolyl)methyl glucosinolates (Heaney and Fenwick, 1980; Shaw et al., 1989), NICI showed the same degree of selectivity toward the detection of glucosinolates as reported under PICI analytical conditions (Shaw et al., 1989). Both analytical techniques were found to give qualitatively similar TIC profiles when a 25-m apolar (HP-1) cross-linked capillary column was used; the base-line separation of 11 per(trimethylsilyl)desulfoglucosinolates (Table I) was the dominant feature. In contrast to PICI, however, the NICI mode proved to be significantly more sensitive. Evaluation of the sensitivity of detection of glucosinolates from rutabaga showed that the GC–NICIMS-based method provided approximately 10-fold better sensitivity for all glucosinolates measured (Table I).

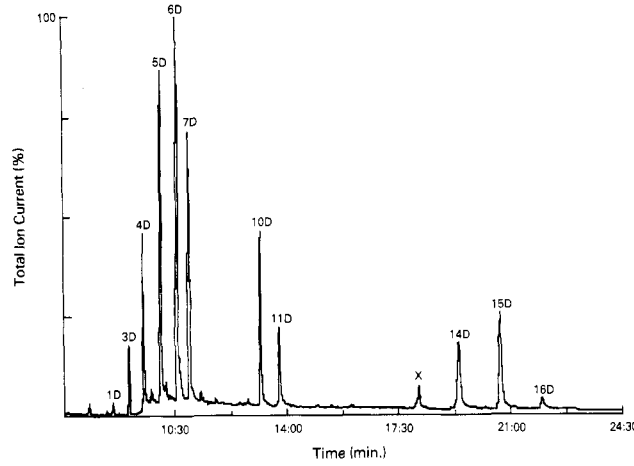


**Figure 1.** Capillary reconstructed TIC chromatogram of per(trimethylsilyl)desulfoglucosinolates from rutabaga. Names of the glucosinolates identified are shown in Table I.

**NICI-Induced Fragmentation of Glucosinolates.** The partial NICI-MS data obtained with methane as the reagent gas are shown in Table I. Under the NICI operating conditions described, silylated desulfoglucosinolates undergo a dissociative electron capture type of fragmentation (Harrison, 1983; Brumley and Sphon, 1987). Indeed, the most diagnostic fragmentation process involved α-cleavage on either side of the thioether group with negative charge stabilization on the sulfur and concomitant loss of either the derivatized side chain or the glucose radical from the molecular negative ion.

An example of this fragmentation is graphically shown in the NICI mass spectrum of pertrimethylsilylated 2-hydroxy-3-butenyl desulfoglucosinolate (6D, Figure 2). A significant proportion of the total ionization was accounted for by four relatively abundant ions at $m/z$ 140, 230, 290, and 483. The presence of ions at $m/z$ 140 and 230 together with the highest mass ion at $m/z$ 483 is a characteristic fragmentation pattern also found in the NICI mass spectrum of every glucosinolate (Table I) and arises from fragmentations associated with the common thioglucose moiety. High-resolution GC–MS analysis of $m/z$ 483 gave an elemental composition of $C_{18}H_{43}O_5SI_4S$ that corresponded to the intact hexakis(trimethylsilyl)-β-D-thioglucose anion and was due to cleavage of the S–C(oxime) bond. The ion at $m/z$ 230 is also a silylated glucose fragment whose elemental composition is $C_9H_{14}O_3SiS$. The likely structure of this fragment is as yet undetermined. The ion at $m/z$ 140 has an elemental composition of $C_6H_5O_2S$, which is consistent with the loss of HOTMS from the daughter ion at $m/z$ 230. A CID MS/MS experiment in which $m/z$ 140 is observed in the daughter ion mass spectrum of $m/z$ 230 supports this suggested fragmentation pathway.

Of more structurally diagnostic value was the relatively stable anion (in this case, $m/z$ 290) formed by the alternative α-cleavage mechanism involving the thioether group, but with charge retention on the side-chain fragment ($R_3C(=NOTMS)S^-$). High-resolution mass mea-

**Table I. Partial Methane NICI Mass Spectra of Selected Per(trimethylsilyl)desulfoglucosinolates**

| glucosinolate | mol wt | $R_3C(=NOTMS)S^-$ | $GLU(TMS)_6S^-$ $m/z$ 483 | $C_9H_{14}O_3SiS^-$ $m/z$ 230 | $C_6H_5O_2S^-$ $m/z$ 140 | other ions |
|---|---|---|---|---|---|---|
| isopropyl (1D) | 641 | 190 (90)[a] | 27[b] | 79[b] | 100[b] | 89 (13), 175 (13), 191 (12), 231 (11), 286 (14), 290 (11) |
| 2-propenyl (2D) | 639 | 188 (83) | 42 | 75 | 100 | 175 (11), 189 (11), 231 (12), 286 (13) |
| 1-methylpropyl (3D) | 655 | 204 (95) | 27 | 80 | 100 | 89 (15), 175 (14), 205 (13), 214 (10), 231 (12), 286 (16), 304 (22), 484 (10) |
| 3-butenyl (4D) | 653 | 202 (94) | 27 | 75 | 100 | 89 (11), 175 (13), 203 (13), 231 (10), 286 (13), 484 (10) |
| 4-pentenyl (5D) | 667 | 216 (98) | 61 | 100 | 87 | 175 (12), 214 (12), 217 (16), 231 (16), 232 (10), 286 (20), 484 (25), 485 (16) |
| 2-hydroxy-3-butenyl (6D) | 741 | 290 (87) | 32 | 82 | 100 | 96 (11), 175 (12), 231 (12), 286 (10), 291 (19), 292 (12), 484 (12) |
| 2-hydroxy-4-pentenyl (7D) | 755 | 304 (100) | 68 | 76 | 48 | 231 (12), 286 (19), 484 (26), 485 (18) |
| 3-(methylthio)propyl (8D) | 687 | 236 (57) | 5 | 59 | 100 | 89 (58), 108 (12), 175 (10), 198 (24), 214 (12) |
| 6-(methylsulfinyl)hexyl (9D) | 745 | 294 (38) | 9 | 60 | 100 | 89 (32), 175 (11), 198 (18), 214 (12) |
| 2-phenylethyl (10D) | 703 | 252 (100) | 35 | 89 | 95 | 89 (11), 175 (10), 214 (12), 231 (14), 253 (19), 286 (17), 484 (13) |
| 5-(methylthio)pentyl (11D) | 715 | 264 (66) | 13 | 73 | 100 | 89 (13), 175 (10), 198 (10), 214 (18), 231 (11), 265 (11) |
| 3-(methylsulfinyl)propyl (12D) | 703 | [c] | | | | |
| 3-(methylsulfonyl)propyl (13D) | 719 | 268 (45) | 7 | 17 | 41 | 146 (100), 236 (12) |
| 3-indolylmethyl (14D) | 800 | 349 (50) | 17 | 77 | 100 | 154 (42), 155 (42), 198 (13), 231 (11), 350 (14) |
| (4-hydroxy-3-indolyl)methyl (15D) | 888 | 437 (33) | 17 | 78 | 100 | 170 (10), 198 (16), 214 (26), 231 (12), 242 (50), 243 (30), 243 (31), 289 (11) |
| (4-methoxy-3-indolyl)methyl (16D) | 830 | 379 (13) | 6 | 12 | 100 | 89 (13), 110 (13), 142 (10), 184 (17), 198 (23), 214 (30), 218 (19), 231 (12) |

[a] $m/z$ (percent relative intensity). [b] Percent relative intensity. [c] Gives multiple peaks by GC.

**Table II. NICI Collision-Induced Daughter Ion Mass Spectra of Selected Per(trimethylsilyl)desulfoglucosinolates**

| glucosinolate | precursor | main daughter ion | | | other |
|---|---|---|---|---|---|
| | | $m/z$ 105 | $m/z$ 89 | $m/z$ 58 | |
| isopropyl | 190[a] | 44[b] | 100[b] | | 73 (4)[c] |
| 1-methylpropyl | 204 | 50 | 100 | 1[b] | |
| 3-butenyl | 202 | 100 | 99 | | |
| 4-pentenyl | 216 | 86 | 100 | | 66 (2), 125 (1) |
| 2-hydroxy-3-butenyl | 290 | 51 | 100 | | |
| 2-hydroxy-4-pentenyl | 304 | 100 | 90 | 2 | 132 (2), 214 (2) |
| 2-phenylethyl | 252 | 100 | 50 | | 134 (2) |
| 5-(methylthio)pentyl | 264 | 100 | 65 | | |
| 3-indolylmethyl | 349 | 100 | 98 | 18 | |
| 4-hydroxy-3-indolylmethyl | 437 | 100 | 91 | | 144 (15), 169 (7), 202 (43), 216 (16) |
| 4-methoxy-3-indolylmethyl | 379 | 31 | 100 | | 303 (28), 354 (16) |

[a] $m/z$. [b] Percent relative intensity. [c] $m/z$ (percent relative intensity).

surements of 6D confirmed the elemental composition of this fragment as $C_{11}H_{24}N_1O_2Si_2S_1$.

CID GC–NICIMS/MS daughter ion spectra of the side-chain anions for all glucosinolates (except 12D) yielded little additional structural information. In spite of the structural diversity found in the glucosinolates investigated in this study, only three stable fragment ions, $m/z$ 105 ($TMSS^-$), 89 ($TMSO^-$), and 58 ($CNS^-$), were readily detectable (Table II). In contrast, PICIMS/MS studies of the side-chain cations (Shaw et al., 1989) proved to be highly diagnostic of the glucosinolate structure. The NICIMS/MS results suggest that even under CID conditions, there exists a strong preference for charge stabilization to occur at only a few electronegative sites common to all the side-chain anions.

Figure 2 shows that there are very distinct differences for these compounds in the extent and type of fragmentation observed under GC–NICIMS or GC–PICIMS conditions with methane as the reagent gas. Although under the relatively more energetic conditions of PICI, the principal bond fission parallels that found under NICI conditions (Shaw et al., 1989), an appreciable array of fragmentation occurs. The main diagnostic side-chain ions, such as $[R_3C{\equiv}NH]^+$ ($m/z$ 170), $[R_3]^+$ ($m/z$ 143), and $[R'CH{=}OTMS]^+$ ($m/z$ 129) (ions observed in the case of the hydroxylalkenyl glucosinolates), and ions arising from the glucose moiety at $m/z$ 451 and 361 (Shaw et al., 1989), also common in silylated methyl glucosides (Richter and Blum, 1983), were not found under NICI conditions. The simpler mass spectra obtained by GC–NICIMS provide useful complementary information. Results (Table I) showed that although no molecular ion is observed under PICI or NICI conditions for any of the silylated desulfoglucosinolates investigated because

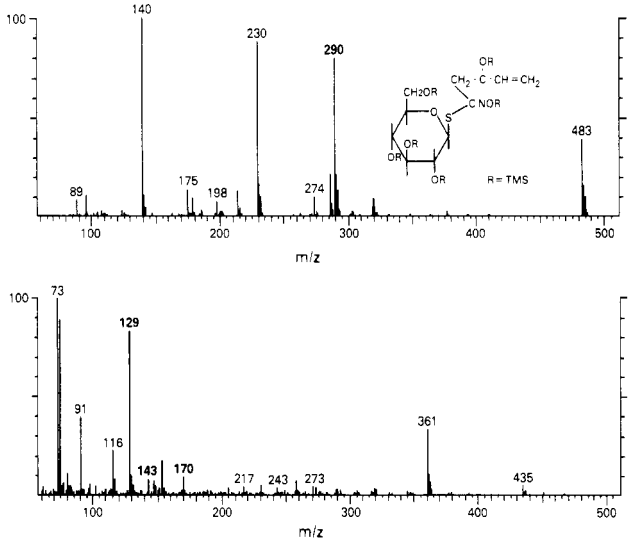


**Figure 2.** NICI (methane) mass spectrum (top) and PICI mass spectrum (bottom) of 2-hydroxy-3-butenyl desulfoglucosinolate; $m/z$ 50–700 range; data background subtracted.

of competitive dissociation processes, NICI data do allow the molecular weights of each compound to be determined indirectly by simple calculation by considering the weights of both the common $m/z$ 483 and side-chain anions. Furthermore, for routine GC–MS screening of *Brassica* vegetable extracts we have found that under NICI conditions, the formation of several major diagnostic ions has proved useful as a highly sensitive method for rapidly detecting the presence of glucosinolates by using mass chromatographic techniques.

In summary, GC–NICIMS using methane as the reagent gas has been found to be more sensitive by 1 order of magnitude, compared with GC–PICIMS, in the detection of glucosinolates isolated from *Brassica* vegetables. In addition, the simple but diagnostically valuable mass spectra obtained with NICI provide a highly selective and reproducible technique that complements GC–PICIMS- and MS/MS-based methods, leading to the rapid detection and characterization of glucosinolate mixtures isolated from *Brassica* vegetables.

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## LITERATURE CITED

Bradfield, C. A.; Bjeldanes, L. F. High Performance Liquid Chromatographic Analysis of Anticarcinogenic Indoles in *Brassica oleracea*. *J. Agric. Food Chem.* **1987**, *35*, 46–49.

Brumley, W. C.; Sphon, J. A. *Applications of Mass Spectrometry in Food Science*; Gilber, J., Ed.; Elsevier Applied Science: London, 1987; pp 141–185.

Christensen, B. W.; Kjaer, A.; Madsen, J. O.; Olsen, C. E.; Olsen, O.; Sorensen, H. Mass Spectrometric Characteristics of Some Pertrimethylsilyl Desulfoglucosinolates. *Tetrahedron* **1982**, *38*, 353–357.

Eagles, J.; Fenwick, G. R.; Heaney, R. K. Gas Chromatography Chemical Ionization Mass Spectrometry of Glucosinolate Derivatives. *Biomed. Mass Spectrom.* **1981**, *8*, 278–282.

Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and Their Breakdown Products in Food and Food Plants. *CRC Crit. Rev. Food Sci.* **1983**, *18*, 123–201.

Harrison, A. G. *Chemical Ionization Mass Spectrometry*; CRC Press: Boca Raton, FL, 1983.

Heaney, R. K.; Fenwick, G. R. The Glucosinolate Content of *Brassica* Vegetables—A Chemotaxonomic Approach to Cultivar Identification. *J. Sci. Food Agric.* **1980**, *31*, 794–801.

Hogge, L. R.; Reed, D. W.; Underhill, E. W. The Analysis of Desulfoglucosinolates Using Thermospray LC/MS. Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO; American Society for Mass Spectrometry: E. Lansing, MI, 1987; Abstr pp 1090–1091.

McGregor, D. I.; Mullin, W. J.; Fenwick, G. R. Review of the Analysis of Glucosinolates. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 825–849.

Mossoba, M. M.; Shaw, G. J.; Andrzejewski, D.; Sphon, J.; Page, S. W. Application of Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectrometry to the Identification of Glucosinolates from *Brassica* Vegetables. *J. Agric. Food Chem.* **1989**, *37*, 367–372.

National Research Council. Inhibitors of Carcinogenesis. In *Diet, Nutrition and Cancer*; National Academy Press: Washington, DC, 1982; pp 358–370.

Richter, W. J.; Blum, W. *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley-Interscience: New York, 1983; pp 423–426.

Shaw, G. J.; Andrzejewski, D. Separation and Identification of Glucosinolates from *Brassica* Vegetables Using High Performance Capillary GC-MS. Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO; American Society for Mass Spectrometry: E. Lansing, MI, 1987; Abstr pp 522–523.

Shaw, G. J.; Andrzejewski, D.; Roach, J. A. G.; Sphon, J. A. Separation and Identification of Glucosinolates from *Brassica* Vegetables Using High-Performance Capillary Gas Chromatography (GC)–Positive-Ion Chemical Ionization Mass Spectrometry (PICIMS) and GC-PICIMS/MS. *J. Agric. Food Chem.* **1989**, *37*, 372–378.

Sosulski, F. W.; Dabrowski, K. J. Determination of Glucosinolates in Canola Meal and Protein Products by Desulfation and Capillary Gas–Liquid Chromatography. *J. Agric. Food Chem.* **1984**, *32*, 1172–1175.

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