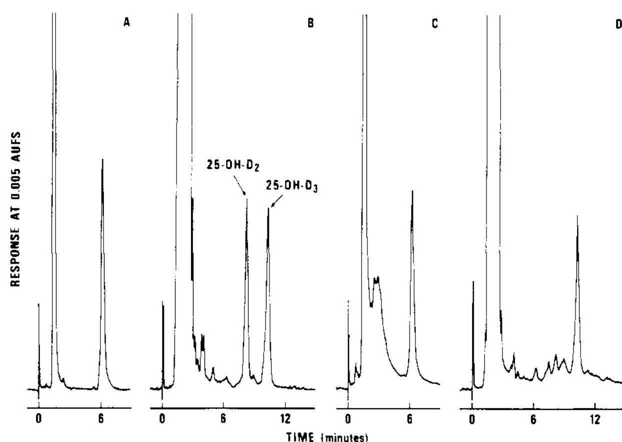

Figure 1. Liquid chromatograms of a mixture of 4.1 ng each of 25-OH-D_2 and 25-OH-D_3 standards (A) and a cow serum extract (C) on the Zorbax ODS column; mixture of 7 ng each of 25-OH-D_2 and 25-OH-D_3 standards (B) and a cow serum extract (D) on the Zorbax Sil column.

Table II. Results of the Same Samples Analyzed for Total 25-OH-D and for 25-OH-D_3 (ng/mL)

total 25-OH-D	25-OH-D_3	difference
61.9	58.6	3.3
58.9	59.5	−0.6
62.4	59.5	2.9
67.2	63.9	3.3
mean 62.6 ± 3.4	60.4 ± 2.4	

mode of operation for the specific determination of 25-OH-D_3. Another difference is that we did not have to use the optional microparticulate silica pre-LC column (Partisil 20) for the cow serum.

Figure 1 shows chromatograms of standard mixtures 25-OH-D_2 and 25-OH-D_3 on the C_{18} bonded silica column (A) and on the nonbonded silica column (B) and cow serum extracts on the bonded (C) and the nonbonded columns (D), respectively. The concentrations in the samples were calculated by comparison of peak height responses of a 25-OH-D_3 standard injected along with the samples. It is possible to calculate the total 25-OH-D concentration from the 25-OH-D_3 standard because 25-OH-D_3 and 25-OH-D_2 have very similar molar absorptivity and therefore comparable response on a UV detector. On both LC systems, the peak height responses were linear to concentration within the range of interest and very reproducible for quantitation. Vitamin D_3 and its known more polar metabolites and trans vitamin D_3 were all well resolved from 25-OH-D_3. Replicate analyses of a pooled cow serum showed a mean concentration of 67.3 ± 5 ng/mL of 25-OH-D_3 (n = 7, range 61.2–73.8 ng/mL). This sample was then analyzed after fortification with an ethanolic solution of 25-OH-D_3 at the levels of 10.3, 20.5, 41.0, and 82.1 ng/mL. The results were 77.0 ± 1.9 (n = 7), 84.9 ± 4.1 (n = 4), 102.8 ± 7.3 (n = 4), and 142.1 ± 9.0 (n = 4) ng/mL at the four levels of fortification representing mean percent recoveries of 94, 86, 87, and 94%, respectively. In another experiment, a serum sample was analyzed 16 times by a new analyst (acknowledgement to K. M. Kennedy, The Upjohn Company). The results showed a range of 43.8–54.1 ng/mL with a mean of 49.7 ± 3.3 ng/mL.

Table II shows results on samples analyzed for total 25-OH-D on the C_{18} bonded micro-silica column and specifically for 25-OH-D_3 on the nonbonded micro-silica column and the differences between the two results. This difference should be the concentration of 25-OH-D_2 in the sample. In this particular sample, the difference was too small for any analytical interpretation.

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Received for review August 20, 1979. Accepted October 17, 1979.

A Semimicro Apparatus for Essential Oil Determination of Multiple Mint Samples by Steam Distillation

An apparatus is described which allows simultaneous steam distillation of ten individual plants for essential oil determination. Volume of recovered oil can be read to 0.01 mL. Tests on mint oil for accuracy and reproducibility gave a mean recovery of 89.9 ± 1.1%. Yields of oil from plant leaves of five species and varieties of mint (*Mentha*) ranged from 3.45 to 8.90%.

The apparatus described in this paper was designed to determine the essential oil content of individual mint (*Mentha* spp.) plants (oil content < 10%, leaf dry weight 3–10 g) grown under controlled environment conditions. The equipment consists of three different parts, a series of distillation units, a frame, and a steam generator. Ten plant samples can be distilled separately at one time.

DESIGN CONSIDERATIONS

Determination of the essential oil content of plant ma-

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Table I. Dry Matter and Oil Yield of Leaves of Five Representative Individual Plants of Species and Varieties of Mint (*Mentha*) Harvested at Flowering Stage

species and variety	dry matter of leaves, g	oil yield mL	oil yield d^a	oil yield g	oil yield %
M. spicata, spearmint	4.47	0.164	0.940	0.154	3.45
M. piperita, peppermint	2.59	0.116	0.917	0.106	4.09
M. cardiaca, Scotch spearmint	7.30	0.520	0.971	0.505	6.92
M. arvensis, var. *piperascens* ("Taiwan") Japanese mint, Taiwan variety	5.72	0.400	0.938	0.375	6.56
M. arvensis, var. *piperascens* So Wo 1 variety	6.34	0.634	0.890	0.564	8.90

[a] Determined at ambient temperature.

terial by steam distillation is a well-established technique based on the Clevenger (1928) apparatus designed to collect oils lighter than water. There are, however, problems with existing apparatus when large numbers of plants are to be assayed on an individual basis. The standard AOAC apparatus (AOAC, 1970) in which condensed water is recycled to the distillation flask is unsuitable for the determination of small quantities of oil because it lacks precision in measuring the oil volume. Furthermore, the oil tends to form droplets which are recycled with the water to the distillation flask making it difficult to know when distillation is complete. Cost is an additional consideration when a number of determinations are to be made at one time.

These problems have been overcome in the apparatus described. The measurement of small volumes of oil (<1.0 mL) is achieved by provision of a section calibrated to 0.01 mL similar to that used by Howe (1956) for the microdistillation of mint leaves. Cost, associated with the need for a number of heating and control units, is minimized by using a single steam generator and a manifold system to distribute the steam to each distillation unit.

CONSTRUCTION

The distillation unit (Figure 1) consists of a two-neck, 500-mL flask (Quickfit Cat. No. FR 500/35/2A) for holding the plant material to be distilled, a condenser (Quickfit Cat. No. C1/12), and a graduated column for collection of the condensate, fitted with a three-way Teflon stockcock (Excelo Cat. No T545/2) for condensate removal. The graduated column consists of a 1-mL and 10-mL graduated Pyrex pipet joined together with the 1-mL pipet above the 10-mL section. The 10-mL section is joined to the stopcock whose sidearm height is so adjusted that when waste condensate water runs off, the level of the collected oil is maintained in the upper section of the 10-mL pipet. A glass rod joining the collection column and steam tube provides rigidity and strength.

Each frame (Figure 2) holds five distillation units. A manifold distributes steam to each unit via an individual needle control valve. The individual valves allow the distillation rate of each unit to be adjusted to approximately 1 mL min^{-1} irrespective of position along the pressurized manifold. Valves may be closed on any unit which may not be required at any time. A separate manifold provides cooling water inlets and outlets for each condenser. Individual flasks are mounted in a moulded plaster of Paris base for support and thermal insulation.

The steam generator is a modified portable steam steriliser (Griffin and George Cat. No. S12-900) of 13-L capacity. A 2-kW electric heating element is sealed into the base of the generator and connected to a pressure switch (Penn P47 AA-1) set to operate at pressures between 27.6 and 34.5 kPa. Steam is taken off at a valve in the upper wall of the generator and fed via pressure tubing to the manifold of the frame assembly.

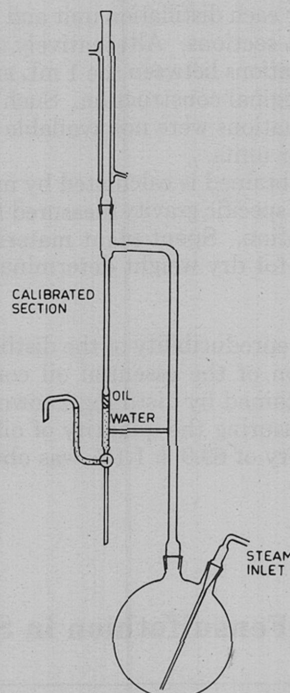

Figure 1. Semimicro steam distillation apparatus for essential oil estimation. Apparatus normally operated with 5° tilt to prevent formation of air locks in narrow calibrated measuring section.

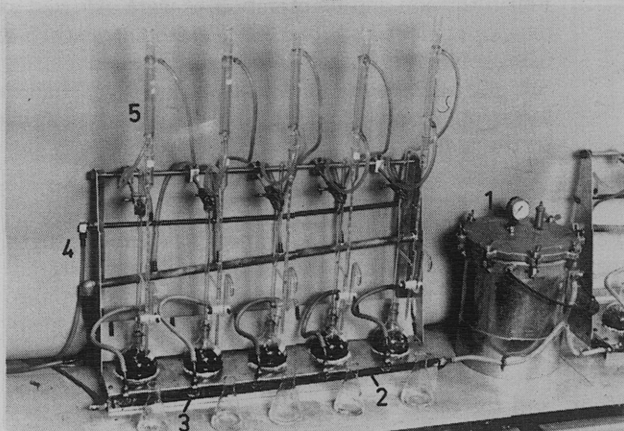

Figure 2. Complete steam distillation assembly comprising: (1) steam generator, (2) steam manifold, (3) needle valve, (4) condenser water manifold, and (5) distillation unit.

OPERATION

For analysis whole plant tops are air-dried in paper bags for 5 days. The leaves are separated from the stems and placed in the sample flask which is then connected to the distillation unit. Steam is injected at the base of the

material and carries the oil up into the condenser. The condenser oil collects on the surface of the condensed water in the lower section of the graduated column. The plant material is distilled for 1.5 h even though most of the oil distills over in 20 min. If the volume of oil collected is less than 1 mL, the level of water in the column may be raised by connecting a water reservoir to the lower outlet of the 3-way stopcock forcing the oil into the calibrated 1-mL section. This permits measurement of the oil volume to 0.01 mL and estimation to 0.005 mL. For oil volumes greater than 1 mL the lower 10-mL section must be used. This results in some loss in precision as graduations are only to 0.1 mL. This lack of precision can be overcome by calibrating individually the junctions of the two calibrated regions for each distillation unit and using both the 10-mL and 1-mL sections. Alternatively a column with continuous graduations between the 1 mL and 10 mL may be used in the original construction. Such columns with continuous graduations were not available at the time of fabrication of our units.

Weight of oil obtained is calculated by multiplying the oil volume by the specific gravity measured for the oil type under determination. Spent plant material is removed after distillation for dry weight determination.

RESULTS

Accuracy and reproducibility of the distillation units in the determination of the essential oil content of mint plants was ascertained by distilling known quantities of mint oil and measuring the quantity of oil collected.

A mean recovery of 89.9 ± 1.1% was obtained on five determinations.

Yields of mint oil varied considerably depending on the species of *Mentha* and the condition of growth of the individual plants. Table I indicates the yields of individual plants of several species and varieties of *Mentha*. This information is provided only as an indication of yields obtained with this apparatus. A more comprehensive account of yields from some of these species will be published in due course.

ACKNOWLEDGMENT

The authors are grateful to the Glassblowing Services, University of Queensland, for fabricating the distillation units and to D. Page-Hanify, Department of Agriculture, for assistance in designing and fabricating the frame and generator.

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Received for review May 15, 1979. Accepted September 4, 1979. This work was in support of a project funded by the Australian Freedom From Hunger Campaign to whom special thanks are extended.

Residues of Fensulfothion in Selected Vegetable Crops

Fensulfothion, *O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate, and three of its metabolites—the oxygen analogue, the sulfone, and the oxygen analogue sulfone—were determined collectively by a simplified method in spinach, table beets, cabbage, and carrots grown in treated soils. Fensulfothion and its metabolites were oxidized with *m*-chloroperbenzoic acid to the single product—the oxygen analogue sulfone. The sulfone was then analyzed as total fensulfothion by gas chromatography using a NP thermionic detector with a nonvolatile rubidium glass bead as the alkali source. Field-treated samples of spinach, table beets, and cabbage had residue levels of <0.01 ppm total fensulfothion. Residues in carrots ranged from 0.15 to 0.60 ppm, depending on the nematicide formulation and rate of application to the soil.

Fensulfothion, *O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate, known as Dasanit, has been registered for use as an insecticide and nematicide on onions, pineapples, pea forage, bananas, sugar cane, sugar beets, peanuts and hulls, rutabagas, tomatoes, and several meat products. Its three metabolites—the oxygen analogue, the sulfone, and the oxygen analogue sulfone—were first detected in cotton plants by Katague and Anderson (1967). Fensulfothion and its three metabolites have also been detected in carrots, cauliflower, and potatoes (Williams et al., 1971) and in corn, grass, and milk (Bowman and Hill, 1971). Fensulfothion and its sulfone have also been detected in muck soil (Williams et al., 1972). Previously the residues of this organophosphate and its metabolites were extracted; the extract was fractionated on a silica gel column; and each fraction was analyzed by gas chromatography with flame photometric detection, Anderson (1973) simplified the determination of fensulfothion and its metabolites by oxidizing with *m*-chloroperbenzoic acid to form one product—the oxygen analogue sulfone. The sulfone was then analyzed by gas–liquid chromatography. This method has been successfully applied to fenthion, disulfoton, and phorate and their metabolites (Bowman and Beroza, 1969).

We undertook to determine the total residue of fensulfothion and its metabolites in selected vegetable crops grown in soils treated for the control of nematodes and evaluate the level of this toxic nematicide.

EXPERIMENTAL SECTION

Solvents and Reagents. Fensulfothion and its oxygen analogue sulfone of 93.8 and 96.0% purity, respectively, were kindly supplied by the Chemagro Corporation, Kansas City, MO. Acetone and chloroform were pesticide-grade solvents from Fisher Scientific, *m*-chloroperbenzoic acid was from Eastman Kodak Co., Hyflo Supercel

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