to that observed with low-tannin sorghum grains. The results obtained here with pepsin digestion support this in vivo observation and suggest that the simple pepsin test described could be useful as a rapid screening procedure for determining the biological value of sorghum grain varieties. Studies are underway to determine the usefulness of this method.

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Received for review July 23, 1979. Accepted October 8, 1979. Contribution from the Purdue University Agricultural Experiment Station, West Lafayette, IN. Journal Paper No. 7677. This research was supported by the U.S. Agency for International Development under a contract entitled "Inheritance and Improvement of Protein Quality and Content in *Sorghum bicolor* (L.) Moench".

Shorter High-Performance Liquid Chromatographic Method for the Determination of 25-Hydroxycholecalciferol in Cow Serum

A short high-performance liquid chromatographic procedure is reported for the analysis of 25-hydroxycholecalciferol (25-OH-D_3) in cow serum. This procedure is faster and requires only 2 mL of serum or plasma as against the 25 g that was required by the procedure that we had reported earlier. In addition, the procedure may be used for the determination of total 25-OH-D (25-OH-D_3 plus 25-OH-D_2) or specifically for 25-OH-D_3.

The primary metabolite of vitamin D_3, 25-OH-D_3, was found useful in reducing the incidence of parturient paresis in cows (Bringe et al., 1971; Olsen et al., 1973a,b). We have reported high-performance liquid chromatographic (LC) procedures for the determination of 25-OH-D_3 in cow blood (Koshy and VanDerSlik, 1976) and in cow liver, kidney, and muscle (Koshy and VanDerSlik, 1977a). Our procedure for cow blood required a large sample size of 25 g of plasma or serum and was lengthy. Besides it was not specific for 25-OH-D_3, as later, when 25-hydroxyergocalciferol (25-OH-D_2) became available, we found that the two compounds had the same retention time on the LC. This problem was resolved by the use of a partition column prior to LC (Koshy and VanDerSlik, 1978). Subsequent to our work on the cow serum, we reported an LC method for 25-OH-D_3 for human serum using 2.5 mL of serum (Koshy and VanDerSlik, 1977b) which could be used either for the determination of total 25-OH-D (25-OH-D_2 plus 25-OH-D_3) or specifically for 25-OH-D_3. This communication is an adaptation of the above method to cow serum.

METHOD

Briefly, the procedure was as follows. (1) Two milliliters of the serum was extracted with 5 mL and 4 mL each of 95% ethanol in a screw-capped centrifuge tube, diluted with 2 mL of water and extracted with 2 × 5 mL of CH_2Cl_2. (2) The extract was evaporated under vacuum and subjected to chromatography on 2 g of silica column (230–400 mesh) using hexane–ether (1:1) and ether–ethyl acetate (9:1) solvent systems. The fraction containing the 25-OH-D was evaporated under N_2. (3) The residue was subjected to partition column chromatography on 0.2 g of Celite 545 using 80:20 methanol–water as the stationary phase and pentane as the mobile phase. (4) For the determination of total 25-OH-D, the extract was subjected to LC on a C_{18} bonded microparticulate silica column. For the specific determination of 25-OH-D_3, the extract was subjected to LC on a nonbonded microparticulate silica column. The LC conditions for the two columns are shown in Table I. For more detailed information of the procedure, the reader is referred to our procedure for human serum (Koshy and VanDerSlik, 1977b).

Table I. LC Conditions

	for total 25-OH-D	for 25-OH-D_3
instrument used	Varian 8500[a]	Varian 8500[a]
column	Zorbax ODS[b] 2.1 mm × 25 cm	Zorbax Sil[b] 2.1 mm × 25 cm
mobile phase	CH_3CN, CH_3OH, H_2O 94:3:3	3% 2-propanol in hexane
flow rate	25 mL/h	45 mL/h
pressure	≈105 atm	≈190 atm
detection	UV-254 nm[c]	UV-254 nm[c]
sample size	5–7 μL	10 μL
sensitivity	0.005 aufs	0.005 aufs

[a] Varian Instrument Division, Palo Alto, CA. [b] DuPont DeNemours, Inc., Wilmington, DE. [c] Waters Model 440, Waters Associates, Inc., Framingham, MA.

RESULTS AND DISCUSSION

The method is simpler than the one we reported before and therefore at least six samples can be analyzed by one analyst in a day. One major difference between our human serum procedure and this one is that a 0.2-g Celite partition column was adequate for both the reversed phase and the adsorption mode of LC. With the human serum, it was necessary to use a 1-g Celite column for the latter

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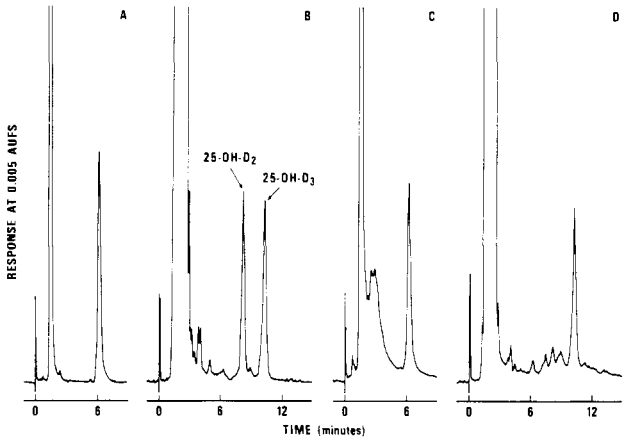

Figure 1. Liquid chromatograms of a mixture of 4.1 ng each of 25-OH-D_2 and 25-OH-D_3 standards (A) and a cow serum extract (C) on the Zorbax ODS column; mixture of 7 ng each of 25-OH-D_2 and 25-OH-D_3 standards (B) and a cow serum extract (D) on the Zorbax Sil column.

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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