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Letter to the Editor

Thin-layer chromatography as an efficient alternative to high-performance liquid chromatography in the assay of plasma vitamin D_3 metabolites

Sir,

The most convenient assay for the determination of vitamin D metabolites (vitamin D here includes both biologically active analogues vitamin D_2 and D_3) in plasma samples is currently a solid-phase extraction using a double Sep-Pak cartridge system (C_{18} and silica) followed by high-performance liquid chromatography (HPLC) and a radioligand binding assay, usually competitive protein binding assay (CPBA) [1]. However, the most useful assays, in the majority of clinical settings, are those for the determination of endogenous 25-hydroxyvitamin D_3 (25-OH-D_3), 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2$-D_3] and 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2$-D_3].

As we have reported previously [2,3], thin-layer chromatography (TLC) on silica gel can quantitatively separate these metabolites. Their elution pattern is similar to that on HPLC [4]. In trying to shorten the above assay, to be useful in routine practice, we have used TLC in place of chromatography on a Sep-Pak silica cartridge and HPLC The recoveries of tritiated vitamin D_3 metabolites added to plasma samples through both conventional and modified assays were compared

EXPERIMENTAL

The following tritiated standards were purchased from the Radiochemical Centre (Amersham, U.K): 25-hydroxy[26(27)-methyl-^{3}H]cholecalciferol (0.43 TBq/mmol), 24,25-dihydroxy[26(27)-methyl-^{3}H]cholecalciferol (2.85 TBq/mmol); 1,25-dihydroxy[26(27)-methyl-^{3}H]cholecalciferol (6.66 TBq/mmol).

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Precoated plates (for nano-TLC) of silica gel 60, 10 cm×10 cm, were from E Merck (Darmstadt, F.R G)

HPLC analysis was carried out with a Model 1090 Hewlett-Packard chromatograph with a diode array detector. The stainless-steel column (25 cm×0.5 cm I.D.) was filled with Spherisorb, 5 μm particle size (Phase Separations Queensferry, U K.) Presep-silica and Presep-C_{18} cartridges were obtained from Tessek (Prague, Czechoslovakia).

The procedure of Reinhardt et al. [5] was modified to extract vitamin D_3 metabolites from plasma samples. Briefly, plasma samples with added tritiated standards were extracted with acetonitrile. The extract was purified on a Presep-C_{18} cartridge to isolate the vitamin D_3 metabolites. The eluate was divided into two aliquots and the first was applied to the silica gel plate, which was developed with chloroform–ethanol–water (183 16 1, v/v) [3]. The second aliquot was, prior to HPLC, purified on a Presep-silica cartridge to separate roughly the vitamin D_3 metabolites. The fractions were chromatographed on a silica gel column with hexane–2-propanol–methanol (94 4 2, v/v) as the mobile phase The combined eluates of the separated tritiated metabolites from both chromatographic systems were monitored for radioactivity

RESULTS AND DISCUSSION

The percentage recoveries of [^{3}H]-25-OH-D_3, [^{3}H]-24,25-$(OH)_2$-D_3 and [^{3}H]-1,25-$(OH)_2$-D_3 added to plasma samples, after the chromatographic procedures either with TLC or HPLC, are summarized in Table I The values show that the fewer steps in the TLC assay can result in higher recoveries than in the HPLC assay.

The reproducibility, accuracy and precision of the assay with TLC (Table II) were tested from the results of recoveries of [^{3}H]-1,25-$(OH)_2$-D_3 added to plasma sample For both intra- and inter-assay tests, they are comparable with these values obtained in the HPLC assay [6].

Therefore, TLC can be used in place of HPLC in the separation of the clin-

TABLE I

RECOVERIES OF VITAMIN D_3 METABOLITES ADDED TO PLASMA SAMPLES

Procedure	Recovery (mean ± S D) (%)		
	[^{3}H]-25-OH-D_3	[^{3}H]-24,25-$(OH)_2$-D_3	[^{3}H]-1,25-$(OH)_2$-D_3
TLC	74 0±4 8	81 5±4 8	75 1±5 3
HPLC	89 0±5 1	55 2±6 1	67 5±4 2

TABLE II

RECOVERIES OF [^{3}H]-1,25-$(OH)_2$-D_3 ADDED TO PLASMA SAMPLES AFTER TLC

Variation	Plasma sample (n=5)	Recovery (mean ± S D) (%)	C V (%)
Intra-assay	A	75 0±5 3	7 0
	B	72 1±7 8	10 8
Inter-assay	C (April 19)	71 4±9 9	13 9
	C (May 13)	69 3±8 9	12 8
	C (June 30)	77 0±6 8	8 8

ically important vitamin D_3 metabolites. The TLC technique appears to be a significant advance for routine assays of these vitamin D_3 metabolites. It will be useful in the diagnosis of a number of diseases that are signified by impaired vitamin D metabolism, and in the control of treatment with calciotropic drugs.

However, there is no doubt that for a detailed study of the metabolism of vitamin D and/or for the measurement of vitamin D_2 analogues, e g. after the administration of vitamin D_2 pharmaceuticals, HPLC is necessary.

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