Journal of Chromatography, 466 (1989) 446–448
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 251

Letter to the Editor

Chromatographic determination of cholesteryl esters synthesized *in vitro*

Sir,

Earlier experiments in this laboratory showed that a cholesteryl ester-synthesizing system present in the cytosol of rat liver is significantly stimulated in rats bearing experimental tumours[1]. Evidence was provided recently[2] that the blood serum of tumour-bearing animals as well as of patients with cancer enhances the production of cholesteryl esters in normal rat liver. For further studies, a method suitable for serial separations of radioactive cholesteryl esters from labelled cholesterol was required.

Thin-layer chromatography (TLC) gives an excellent separation of both these lipid classes but the procedure is rather complicated and time-consuming[3]. The method now described was developed for a simple and rapid separation of labelled cholesteryl esters from radioactive cholesterol (which is used as the starting material for the subcellular synthesis of esters). This procedure shows a good reproducibility and is useful for serial analyses.

[1a,2a(n)-^{3}H]Cholesterol (50 Ci/mmol) was a product of Amersham (Amersham, U.K.). Cholesteryl 14-methylhexadecanoate and [^{3}H]cholesteryl 14-methylhexadecanoate (50 Ci/mmol) were synthesized as described by Helmich and Hradec[4]. Separations of cholesteryl esters from cholesterol were performed on thin layers of silica gel G (Merck, Darmstadt, F.R.G.) developed with *n*-hexane–diethyl ether (95:2, v/v). Mixtures for the cell-free synthesis of cholesteryl esters contained rat liver cytosol and [^{3}H]cholesterol as described in detail elsewhere[2].

The novel procedure for the determination of newly formed radioactive cholesteryl esters included two steps. (1) Portions (100 μl) of the cytosol after the incubation were applied to strips of Whatman 31ET (2 cm × 2 cm) filter-paper and dried under an IR lamp. The dried filters were placed in 3 ml of ethanol–diethyl ether (1:2, v/v) in glass tubes, the tubes were stoppered and left for 60 min at room temperature with occasional shaking. The filters were then removed and washed with *ca.* 1 ml of the extraction mixture. The pooled extracts were evaporated to dryness at 70°C under a stream of nitrogen. (2) Residues were dissolved in 100 μl of chloroform and 75-μl portions were applied to microcolumns of silica gel (Silica-cart) obtained from Tessek (Prague, Czechoslovakia). Plastic syringes attached to the microcolumns were filled with 5.0 ml of *n*-heptane–diethyl ether (90:10, v/v) and the columns were eluted using a moderate pressure. The eluate was collected directly in scintillation vials containing a toluene-based scintillation mixture and the radioactivity (dpm) was assayed using a Beckman 5801 liquid scintillation system.

Analyses of lipids require mostly extraction by conventional methods[5]. These procedures are time-consuming and losses of material may occur during the extraction of small samples. Mazière *et al.*[6] overcame this difficulty by drying suspensions of

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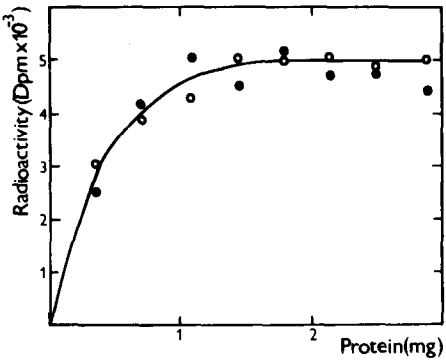

Fig. 1. Comparison of liquid–liquid extraction of samples with the extraction of dried samples on filters. Increasing quantities of rat liver cytosol were incubated with [^{3}H]cholesterol as described[2] and mixtures were extracted by the method of Folch *et al.*[5] (●) or as described (○). Extracts were then separated on microcolumns of silica gel.

TABLE I

RECOVERY OF CHOLESTERYL ESTERS AFTER CHROMATOGRAPHY ON MICRO-COLUMNS OF SILICA GEL

All samples were chromatographed in duplicates on microcolumns of silica gel as described. Quantitation of cholesteryl esters was performed as described by Burke *et al.*[8].

Ester	*Input (mg)*	*A_{550} units*		*Recovery (%)*
		Before chromatography	*After chromatography*	
14-Methylhexadecanoate	0.5	0.057, 0.061	0.060, 0.055	97.5
	1.0	0.130, 0.125	0.118, 0.321	98.0
	2.0	0.243, 0.251	0.210, 0.217	86.4
Stearate	0.5	0.067, 0.055	0.055, 0.058	92.6
	1.0	0.123, 0.117	0.114, 0.098	88.3
	2.0	0.235, 0.239	0.206, 0.222	90.3

cultured cells directly on thin layers used for the subsequent separation by the usual TLC and reported recoveries comparable with usual extraction methods. For our purposes, only the separation of two lipid classes was required and extraction of the material dried on filter-papers was found preferable. Recoveries obtained for cholesteryl esters using this technique were comparable with those obtained by the usual extraction procedure of Folch *et al.*[5] (Fig. 1).

Microcolumns of silica gel were found very useful for a rapid and efficient separation of radioactive cholesteryl esters from labelled cholesterol. Similar micro-columns were used for the separation of polar from non-polar lipids[7]. Recoveries of radioactive cholesteryl esters obtained after chromatography on silica microcolumns (Table I) were in good agreement with those after TLC.

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(First received September 19th, 1988; revised manuscript received December 27th, 1988)