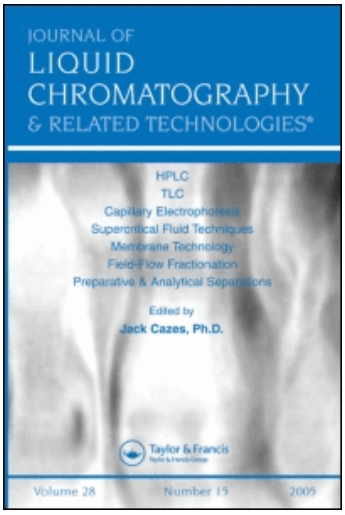

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Shetty, Prabhakara H. , Park, Yoko Y. , Fried, Bernard and Sherma, Joseph(1991) 'HPLC Determination of Cholesterol Esters in the Digestive Gland-Gonad Complex of Biomphalaria Glabrata Snails Fed Hen's Egg Yolk Versus Leaf Lettuce', Journal of Liquid Chromatography & Related Technologies, 14: 4, 643 — 649
To link to this Article: DOI: 10.1080/01483919108049276
URL: http://dx.doi.org/10.1080/01483919108049276

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(4), 643-649 (1991)

HPLC DETERMINATION OF CHOLESTEROL ESTERS IN THE DIGESTIVE GLAND-GONAD COMPLEX OF BIOMPHALARIA GLABRATA SNAILS FED HEN'S EGG YOLK VERSUS LEAF LETTUCE

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ABSTRACT

HPLC was used to analyze cholesterol esters in the digestive gland-gonad (DGG) complex of Biomphalaria glabrata snails fed leaf lettuce or hen's egg yolk. The lettuce and yolk were also analyzed for cholesterol esters. Trace amounts of cholesteryl linolenate and/or arachidonate were found in lettuce and the DGG of snails fed lettuce. Cholesteryl oleate, cholesteryl arachidonate and/or linolenate, cholesteryl palmitate, and cholesteryl linoleate were the major cholesterol esters in both egg yolk and the DGG of yolk-fed snails, but the percentage composition of each ester was markedly different in both populations.

INTRODUCTION

Recent thin-layer chromatography (TLC) studies on the effects of dietary-induced hyperlipidemia on the digestive gland-gonad (DGG) complex of the planorbid snail Biomphalaria glabrata have documented increases in several neutral lipid

fractions of snails fed hen's egg yolk versus controls maintained on leaf lettuce [1]. Attempts to determine the effects of a high lipid diet on the cholesterol ester fraction of the DGG were equivocal. Using the Mangold solvent system, the cholesterol ester fraction moved at or near the solvent front and was contaminated with other non-polar compounds such as wax esters and hydrocarbons.

In the present study, preparative TLC and column high performance liquid chromatography (HPLC) were used to analyze the cholesterol ester fraction of the DGG of B. glabrata snails maintained on either hen's egg yolk or leaf lettuce. HPLC analysis of cholesterol esters in egg yolk and leaf lettuce is also reported.

EXPERIMENTAL

Sample preparation

Stock cultures of B. glabrata snails were maintained as previously described (1). Snails with a shell diameter of 10-15 mm were fed ad libitum for one week on a leaf lettuce or boiled hen's egg yolk diet. DGG fractions weighing 200 mg and 400 mg (wet weight) were collected from pools of 5-10 yolk-fed and lettuce-fed snails, respectively, and lipids were extracted with chloroform-methanol (2:1) and non-lipid material removed by Folch wash (0.88% KCl) as described earlier (1). The lipid-containing organic phase was separated and evaporated to dryness under a stream of nitrogen gas at

TABLE 1

HPLC Retention Times (Minutes) of Cholesterol Esters

Cholesteryl linolenate	16.0
Cholesteryl arachidonate	16.6
Cholesteryl linoleate	20.6
Cholesteryl palmitoleate	21.4
Cholesteryl myristate	23.3
Cholesteryl oleate	27.7
Cholesteryl palmitate	30.4
Cholesteryl heptadecanoate	35.1
Cholesteryl stearate	40.4

room temperature. Hen's egg yolk and leaf lettuce samples (0.1-0.2 g and 1 g, respectively) were extracted and the extracts cleaned-up and evaporated in the same manner.

Standard solutions

The cholesterol esters studied are listed in Table 1. Standards were obtained from Sigma Chemical Co. or Matreya, Inc. Individual and mixed standard solutions were prepared at a concentration of 1.0 ug/ul of each ester in chloroform.

Preparative TLC

TLC samples were prepared by reconstituting the extract residue with 500 ul, 200 ul, 500 ul, and 500 ul for yolk-fed DGG, lettuce-fed DGG, yolk, and lettuce samples, respectively. The entire amount of each sample was streaked across the origin of a 20 x 20 cm Analtech preparative silica gel GF plate (500 microns layer thickness) using a 25 ul

Drummond digital microdispenser. A cholesteryl oleate standard solution was applied on each side of the sample streak. Plates were developed in a Mangold solvent system modified to reduce R_f values, petroleum ether (35-60 ^{o}C)-diethyl ether-glacial acetic acid (80:20:2). Cholesterol esters were recovered by scraping the sample band with chromatographic mobility identical to that of the cholesteryl oleate standard and eluting with chloroform. The eluate was evaporated to dryness under a stream of nitrogen at room temperature.

HPLC

The cholesterol ester fraction from PTLC was dissolved in 200 ul of chloroform, and 20 ul was injected into a Spherisorb S5 ODS-2 column (25 cm length and 4 mm diameter) attached to a Waters M-45 solvent delivery system through a Rheodyne 7125 injector with 20 ul sample loop. Cholesterol esters were monitored at 212 nm using a Waters 481 LC UV detector. The mobile phase was acetonitrile-isopropanol (6:4) (2) with a flow rate of 1.7 ml/minute. A Hewlett-Packard HP 3394 integrator was used to record chromatograms. Cholesterol esters were identified by comparison of retention times of peaks in sample chromatograms with those from a standard mixture containing 1.0 ug/ul of each ester, with confirmation by co-chromatography of sample spiked with the standard(s) thought to be present. Percent composition was estimated by use of sample peak measurements and response factors calculated from the standard chromatogram.

TABLE 2

Cholesterol Esters Determined by HPLC in Hen's Egg Yolk and the DGG of Egg Yolk-Fed B. glabrata Snails

	% of total cholesterol esters	
	egg yolk	DGG
Cholesteryl oleate	32.8	54.8
Cholesteryl palmitate	19.3	15.7
Cholesteryl arachidonate and/or cholesteryl linolenate	32.0	14.7
Cholesteryl linoleate	15.8	8.7
Cholesteryl palmitoleate	-	6.1
Cholesteryl stearate	trace	-

RESULTS

Table 1 lists the retention times of the cholesterol esters using the HPLC parameters described above. Although cholesteryl linolenate and arachidonate could be partially separated in the standard mixture, they were not resolved well enough in sample chromatograms, which had somewhat broader peaks, to distinguish between these two compounds.

Table 2 lists the cholesterol esters found in egg yolk and the DGG of yolk-fed B. glabrata, with their percentage composition. Cholesteryl linolenate and/or arachidonate were the only esters detected in leaf lettuce and the DGG of snails maintained on a leaf lettuce diet, and these were present at trace levels.

DISCUSSION

The literature contains no information on cholesterol ester content in planorbid snails. Results of our study show

that cholesterol ester content in the DGG of Biomphalaria glabrata snails is influenced by dietary intake. The major fatty acid in leaf lettuce is linolenic acid [3]. Results of our study show trace amounts of cholesteryl linolenate and/or arachidonate in both leaf lettuce and the DGG of B. glabrata fed leaf lettuce.

Bitman and Wood [4] analyzed cholesterol esters in hen's egg yolk by GLC and found cholesteryl oleate (46%), cholesteryl linoleate (34%), cholesteryl palmitate (15%), cholesteryl palmitoleate (4%), and cholesteryl stearate (2%). Our HPLC analysis of hen's egg yolk also found significant amounts of cholesteryl oleate, linoleate, and palmitate.

Most of the cholesterol esters found in egg yolk were also present in the the yolk-fed snail DGG, but the percentage composition of each ester was markedly different in the two samples. Although dietary fat intake influences the cholesterol ester composition of the snail DGG, other influences (presumably metabolic) also play a role.

ACKNOWLEDGEMENT

P.H.S. was a Dreyfus teaching/research fellow in the Chemistry Department. Y.Y.P. was supported with funds from the Dreyfus grant. This research was supported in part by NIH AREA grant TMP (AHR-F)1R15HL40441-01.

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