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DETERMINATION OF CHOLESTEROL AND CHOLESTERYL ESTERS IN SERUM BY LC

Consumers in the US are inundated with news stories and advertisements on the need for a low cholesterol intake in their diets. Presumably, this low cholesterol intake will reduce the risk of arterosclerosis and other cardiovascular diseases. Due to the possible deleterious health effects of this compound and its related esters, a simple, fast method for their determination should be useful to a large number of researchers. A recent report (1) suggests using a non-aqueous reverse-phase LC method for their determination in human serum. Previously, cholesterol determination was done by a laborious and time-consuming gas or thin-layer chromatography method. The LC method provides a practical and rapid approach for cholesterol analysis.

Analysis of the cholesterol was done on a Waters™ LC equipped with a Radial-Pak™ cartridge containing 10µ Resolve™ C_{18} packing material (10 cm X 8 mm ID) in an RCM-100® Radial Compression Module. The mobile phase consisted of acetonitrile/isopropanol (45/55) v/v at 2.0 ml/min with UV detection at 210 nm. Figure 1A is a chromatogram of free cholesterol and cholesteryl ester standards; Figure 1B is an extracted serum sample showing the free cholesterol and its associated cholesteryl esters.

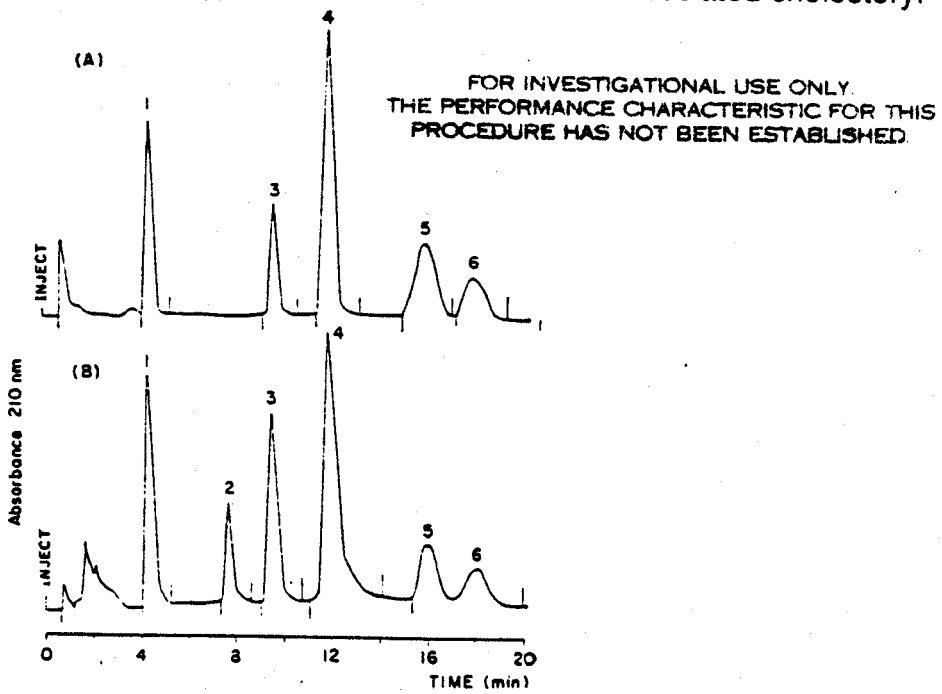

Figure 1. Chromatogram (A) represents free cholesterol and cholesteryl ester standards. Chromatogram (B) represents sample of serum. Peak 1; free cholesterol, 2; unidentified, 3; cholesteryl arachidonate, 4; cholesteryl linoleate, 5; cholesteryl oleate, 6; cholesteryl palmitate.

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In order for this procedure to be used on serum samples, a modified extraction procedure based on the work of Peter and Reynolds (2) is used. In this analysis (1), 0.2 ml of serum is added to 5.0 ml of a solution of isopropanol and 0.75M NaOH (33/17 by volume) and vortexed for 15 seconds. After 2 minutes, 2 ml of n-octane is added and vortexed again for 60 seconds. After brief centrifugation at 400X g, a 1 ml aliquot of the n-octane (upper) layer is pipetted into a 13 X 100 mm glass test tube and evaporated with a gentle stream of N_2. The residue is taken up in 1 ml of isopropanol, and a 50µl aliquot is injected into the LC.

In describing any analytical method, precision and analytical recovery are important factors to be considered. Table I shows the low variation in the LC method for a 5-day period. Table II shows analytical recoveries obtained after running through the entire procedure. Recoveries ranged from 95% to 105%.

Table I

Within-run and between-run precision of cholesterol estimation

Cholesterol	Within-run (n=10)*		Between-run (n=10)**	
	Mean±SD (mg/100 ml)	CV (%)	Mean±SD (mg/100 ml)	CV*** (%)
Free cholesterol	36.7±1.50	4.1	37.0±1.92	5.2
Cholesteryl palmitate	19.9±1.51	7.6	19.7±1.60	8.1
Cholesteryl oleate	28.1±0.84	3.0	28.8±1.27	4.4
Cholesteryl linoleate	73.3±2.71	3.7	72.5±4.13	5.7
Cholesteryl arachidonate	13.2±0.83	6.3	13.1±0.92	7.0

* For within-run analysis, ten aliquots of a single serum sample were extracted and carried through the entire analysis by HPLC.

** For between-run analysis, duplicate aliquots of a serum pool were extracted and analysis by HPLC over a five-day period.

Table II

Analytical recovery of the cholesterol standards

Cholesterol	Standard in IPA (mg/100 ml)	Observed (n=4) (mean, mg/100 ml)	Recovery (%)
Free cholesterol	25.0	26.3	105
Cholesteryl palmitate	25.0	24.8	99
Cholesteryl oleate	25.0	25.5	102
Cholesteryl linoleate	25.0	24.3	97
Cholesteryl arachidonate	25.0	23.8	95

A final interesting aspect of this study reported by the authors is that this same analysis may be run on a µBondapak™ C_{18} column (30 cm X 3.9 mm ID) at the same flow, with only changing the mobile phase to 60/40 (v/v) acetonitrile/isopropanol, with the same resulting chromatogram.

As cholesterol and its related compounds are being indicated as the culprits of cardiovascular diseases, LC appears to be a good choice for monitoring these ubiquitous compounds in serum.

1. Kim, J. C., and Chung, T. H., *Korean J. Biochem.* 16 (1984) 69-77.
2. Peter, F., Reynolds, R. G., *J. Chromatogr.* Biomed Appl. 143 (1977) 153-160.