

## A sensitive microgel filtration method to analyze the lipoprotein profile of individual serum samples on a ÄKTAbasic 10 chromatography system

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**Improved methods for the analysis of VLDL (very-low-density lipoprotein), LDL (low-density lipoprotein), and HDL (high-density lipoprotein) cholesterol; and triglyceride lipoproteins in animals are described. Using ÄKTAbasic™ 10 and Superose™ 6 PC 3.2/30 columns, we were able to reduce sample size to 5 to 10 µl of diluted plasma or serum, which enables analysis of individual animals. In addition, this method reduces by 10-fold both mobile phase and reagent consumption in comparison with analysis using classical FPLC™ system.**

### Introduction

Gel filtration is the method of choice for the separation of lipoproteins. This article describes the use of Superose 6 PC 3.2/30 columns, which have a very small internal diameter (3.2 mm). The reduction of the internal diameter from 10 mm, (e.g., Superose 6 10/300 GL) to 3.2 mm represents a 10-fold reduction of the column volume. To maintain the same resolution, the flow rate must be decreased by the same factor, allowing a 10-fold increase in the UV detection sensitivity.

### System setup

ÄKTAbasic 10 chromatography system and a Superose 6 PC 3.2/30 column were used. An online filter (2 µm, Interchim) was installed before the column.

The flow rate of the pumps was set at 100 µl/min, with 50% for pump A and 50% for pump B. One pump was used for the mobile phase, the other for the enzymatic reagent. The real flow rate through the column was 50 µl/min. All tubing was blue PEEK (0.010 in I.D., 1/16 in O.D.). The enzymatic reagent was mixed with the eluent at the end of the column in a low-dead volume T-connector. The mixture entered 8 m of tubing, of which 5 m was submerged in a waterbath controlled at 37°C. Tubing lengths from the T-connector to the waterbath, and from the waterbath to the UV detector were the same (1.5 m). The UV detector was set at 500 nm. No back pressure regulator was used.

The phosphate buffer mobile phase (0.01 M phosphate, 0.138 M NaCl, 0.0027 KCl, pH 7.4, 0.2 mM EDTA) was filtered (0.22 µm) prior to use.

The system was tested at room temperature and at 4°C. The temperature has no influence on the resolution (results not shown). Analyses were performed at 4°C with an operating pressure of 1.2 MPa. The pressure alarm was changed to 1.5 MPa, which does not affect the life span of the column.

Reagents from Roche (cholesterol CHOD-PAP and triglycerides GPO-PAD) were used. These reagents

remained in solution, whereas others precipitated and clogged the tubing.

Sera were diluted 1- to 2-fold with the mobile phase in 300 µl microvials (AIT). In general, 5 to 10 µl were injected by a partial loopfill method. Vials containing mobile phase were used for the flush volume.

All animals came from Charles River France except the fat-fed Golden Syrian Hamster serum, which was supplied by the CERB.

### Results

Table 1 summarizes the parameters used with the classical FPLC methods, as described by Garber *et al.* (1), and the scaled-down method described above.

As expected, the reduction of the column diameter allows for low flow rates (from 400 µl/min to 50 µl/min). The ÄKTAbasic 10 system is able to work at 25 µl/min, but 50 µl/min was chosen to achieve a 50 min runtime. The human lipoprotein profile is shown in Figure 1, and no decrease in the resolution in comparison with those obtained by classical FPLC methods was observed.

As little as 1.4 mg/dl of HDL cholesterol can be measured (Fig 2). This represents an injected quantity of 140 ng of HDL on the column which reveals the sensitivity of this method. Moreover, the increase in the sensitivity reduces the amount of sample required (5-10 µl of diluted plasma rather than pure plasma).

Individual hamster sera, mouse sera, and rat plasma can be analyzed with this method (Figs 3, 4, 5 respectively). The comparison between the individual profiles reveals dramatic differences, and the use of individual samples can increase the accuracy of the results in comparison with the use of pooled samples.

This system setup can also be used with a reagent for triglyceride dosage (2). It detects glycerol obtained after hydrolysis of the triglycerides. The profile of diluted rat serum is shown in Figure 6.

### Conclusion

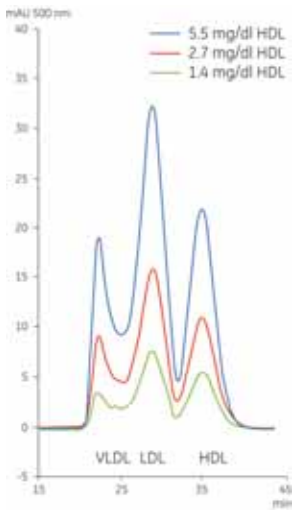
The Superose 6 PC 3.2/30 gel filtration column can be used to analyze lipoprotein profiles. The small column reduces the amount of sample to 5-10 µl of diluted (1:1 or 1:2 v:v) plasma or serum, allowing the analysis of individual animals.

### References

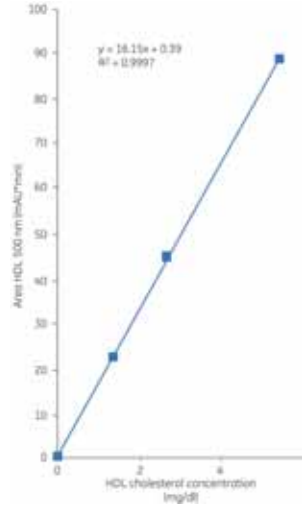
1. Garber D. W. *et al.* A sensitive and convenient method for lipoprotein profile analysis of individual mouse plasma samples. *J. Lipid Res.* **41**, 1020–1026 (2000).
2. Hara, I. *et al.* High-performance liquid chromatography of human serum lipoproteins: selective detection of triglycerides by enzymatic reaction. *J. Chromatogr.* **239**, 549–557 (1982).

Table 1. Comparison of key parameters used with classical FPLC and the scaled-down method developed in this report

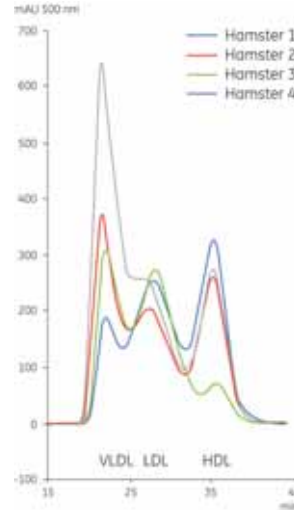
	Classical FPLC method (1)		Scaled-down method on ÄKTAbasic 10
Superose 6 column	300 mm x 10 mm	300 mm x 10 mm	300 mm x 3.2 mm
Column flow rate	400 µl/min	400 µl/min	50 µl/min
Reagent flow rate	fractions	200 µl/min	50 µl/min
Sample	200 µl	10 µl	5-10 µl of 1:1 or 1:2 diluted serum or plasma
Elution volume	24 ml	24 ml	2.5 ml
RunTime	60 min	60 min	50 min



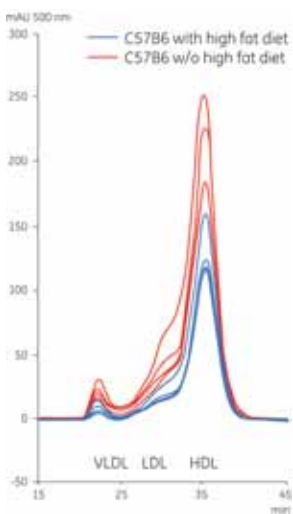
**Fig 1.** Different dilutions of human lipoproteins with internal HDL cholesterol standard (LabBo Immunosystems); injection volume: 10 µl.



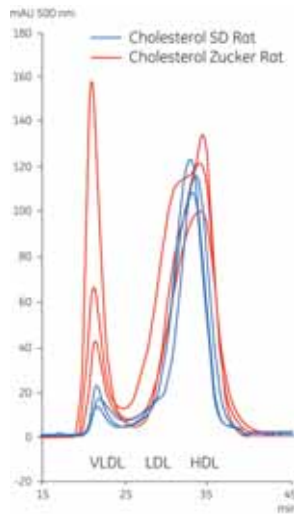
**Fig 2.** Calibration curve for HDL cholesterol; injection volume: 10 µl.



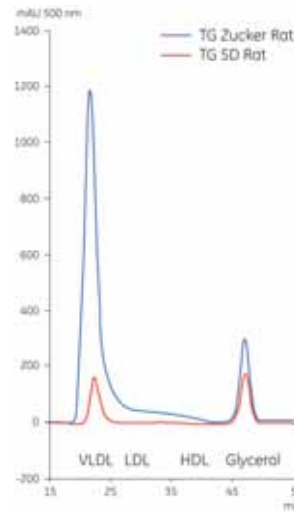
**Fig 3.** Individual lipoprotein profile of fat fed Golden Syrian Hamsters; injection volume: 10 µl of diluted serum in mobile phase 1:2 (v:v).



**Fig 4.** Individual lipoprotein profile of C57B6 mice fed normal and fat diet; injection volume: 10 µl of diluted serum in mobile phase 1:2 (v:v).



**Fig 5.** Individual lipoprotein profile of Sprague Dawley and Zucker rats for cholesterol detection; injection volume: 10 µl of diluted serum in mobile phase 1:1 (v:v).



**Fig 6.** Individual lipoprotein profile of Sprague Dawley and Zucker rats for triglyceride detection; injection volume: 10 µl of diluted serum in mobile phase 1:1 (v:v).

## Ordering information

Product	Code number
Superose 6 PC 3.2/30 *	17-0673-01
ÄKTAbasic 10	18-1401-00
Precision Column Holder	17-1455-01

\* PC columns are optimized for use with SMART systems. PC columns require the use of a Precision Column Holder when used with chromatography systems other than SMART systems.