

## Determination of Sterols in Dairy Products and Vegetable Fats by HPLC and GC Methods

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**Abstract:** Cholesterol concentrations in goat milk, goat milk cheeses, ewe's milk, ewes milk cheeses, dairy bioproducts, and concentrations of cholesterol, stigmasterol and sitosterol in butter, butter with added vegetable fats and margarines were evaluated by RP HPLC method. Parallel analyses by capillary GC were performed. Prior to the final chromatographic analyses the saponification step was used, followed by the extraction of the unsaponifiable residue into *n*-hexane. Parameters of RP HPLC method were compared with parameters of GC determination. The detection limits (LOD) determined on the bases of blank samples analysis were 5.2 mg/kg for cholesterol, 4.8 mg/kg for stigmasterol and 14.7 mg/kg for sitosterol. Recovery ranged between 80–92%, repeatability expressed as RSD of 12 parallel samples measurements was 4.2–6.8%. Accuracy tested on the SRM 1845 Whole Egg Powder (NIST) was 95.7%.

**Keywords:** cholesterol; sterols; milk; dairy products; RP HPLC; GC

### INTRODUCTION

Cholesterol, sitosterol and stigmasterol are polycyclic steroid compounds with similar chemical structure. Cholesterol is a typical animal sterol, e.g. its content in milk fat is 95–98%, stigmasterol and sitosterol are referred to as phytosterols. Both cholesterol and phytosterols occur in free and esterified forms (CARERI *et al.* 2001).

The determination of sterols in dairy products is carried out for the following objectives: to measure the total cholesterol content to obtain nutritional information and to detect the presence of vegetable fats (CARERI *et al.* 2001; CONTARINI *et al.* 2002).

The most appropriate and frequently used method for the determination of total sterols content in foods is the direct saponification, followed by the extraction of the unsaponifiable residue into the nonpolar solvent and final gas chromatographic detection. Liquid chromatographic methods are not so frequently used (FENTON 1992; ABIDI 2001).

The aim of our study was to develop simple, fast and reliable RP HPLC method alternative to

the gas chromatography and normal phase liquid chromatography for the determination of total cholesterol and phytosterols concentrations in milk and dairy products.

### MATERIAL AND METHODS

**Samples.** The content of total cholesterol was monitored in samples of goat's and ewe's milk, in goat's and ewe's cheeses and in dairy bioproducts. The concentrations of cholesterol, stigmasterol and sitosterol were determined in samples of fresh butter, butter with added vegetable fat and margarines. Samples of goat and ewes milk were obtained from the farms in the Moravia region, samples of butter, vegetable fats and dairy bioproducts from the shops in the Czech Republic.

**Chemicals and standards.** Cholesterol (grade I, approx. 99%, Sigma-Aldrich, USA) stigmasterol (approx. 95%, Sigma-Aldrich, USA),  $\beta$ -sitosterol (40%, Sigma-Aldrich, USA, and 80%, Extra Synthèse, France), methanol, *n*-hexane, isopropanol (Merck, Germany), fenoltalein, potassium hyd-

roxide, sodium sulphate (Penta, CR). All chemicals were p.a. or HPLC grade. Standard reference material 1845 (Cholesterol in Whole Egg Powder, NIST, USA) was used.

**Sample preparation.** To the 1 g of the sample methanolic solution of 10 mol/l potassium hydroxide (9:1) was added and refluxed for 30 minutes. To the samples of milk and dairy products 0.1% solution of stigmasterol as internal standard was added. The samples of butter or fat were dissolved in 10 ml of isopropanol prior to the saponification and an aliquote of 1 ml was used. After cooling 5 ml of deionised water and 10 ml of *n*-hexane were added and intensively shaken for 20 minutes. The organic layer was separated, washed with deionised water to the neutral reaction and dried with sodium sulphate. The hexane solution was directly analysed by gas chromatography and from an aliquot of about 5–7 ml hexane was evaporated and residue dissolved in 1 ml of methanol for HPLC analysis.

**HPLC determination.** Analytes were determined by the reverse phase HPLC on Zorbax Eclipse XDB C8, 150 × 4.6 mm, 5 µm (Agilent, USA) column was used. Analyses were performed on the liquid chromatograph Alliance 2695 (Waters, USA) with

PDA 2996 detector at 205 nm. Isocratic elution with mobile phase of methanol and water (95:5) mixture at flow rate 0.7 ml/min was used. Column temperature was set up at 35°C, injection volume was 10 µl. Data were collected and evaluated by software Empower (Waters, USA). Examples of HPLC chromatogram are in Figures 1 and 2.

**Gas chromatography determination.** Analyses were performed on the 6820 GC System (Agilent, USA) chromatograph with flame ionisation detection on the capillary column DB 1, 15 m × 0.53 mm, film 0.5 µm (J&W Scientific, USA).  $T_{inj}$  250°C,  $T_{det}$  300°C, linear gradient 50–260°C. Flow rate of carrier gas  $N_2$  was 3 ml/min, splitless injection. Data were collected and evaluated by software Clarity (DataApex, CR).

## RESULTS AND DISCUSSION

Each sample was analysed at least at duplicates, with each series blank samples were performed. The internal standard method for the determination of cholesterol in milk and dairy products and the external standard method for the determination of cholesterol, stigmasterol and sitosterol in

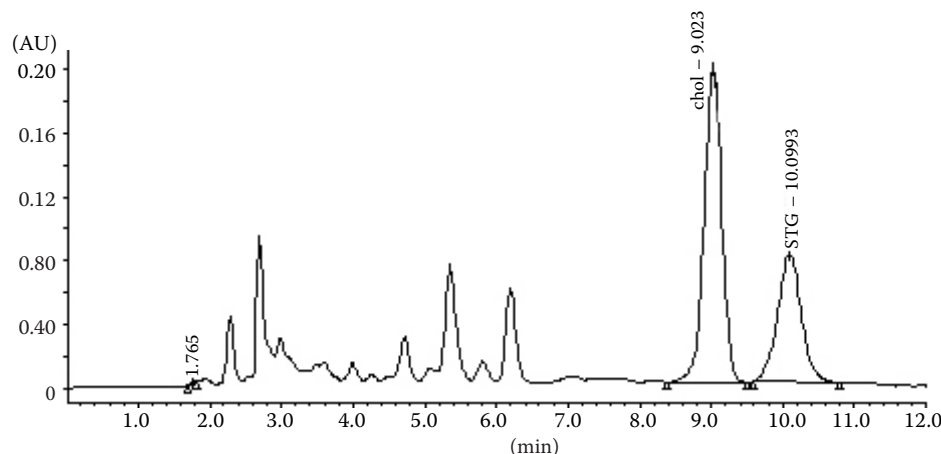


Figure 1. RP HPLC chromatogram of cholesterol and stigmasterol (internal standard) in goat milk cheese

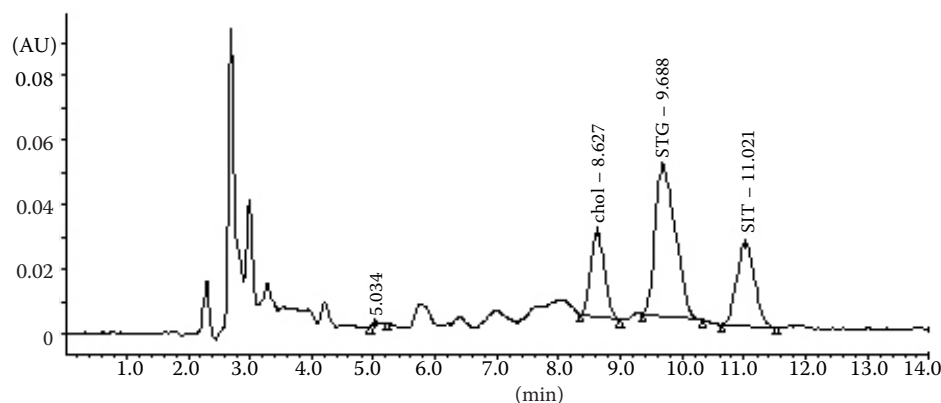


Figure 2. RP HPLC chromatogram of butter with added vegetable fat

Table 1. Cholesterol contents (mg/kg) in goat's milk, goat's cheeses, ewe's milk and ewe's cheeses

Samples	Range	Average value
Raw goat's milk ( $n = 40$ )	117.0–192.1	150.3
Goat's cheese, fresh ( $n = 24$ )	60.2–69.2	650.6
Goat Eidamer, hard cheese ( $n = 9$ )	649.6–879.1	745.8
Ewe's milk ( $n = 12$ )	241.6–275.8	264.0
Ewe's cheese ( $n = 18$ )	487.1–754.9	586.1

Table 2. Cholesterol contents in BIO dairy products

Samples	Cholesterol (mg/kg)	RSD (%)
AM BIO Blue-veined cheese	503.8	8.0
BIO Hofdammer, natural hard cheese	503.5	2.7
BIO Ementaler, hard cheese	583.6	3.8
BIO Gouda	553.9	2.1
BIO ice cream	256.4	6.8
BIO fresh milk	126.0	0.2
BIO yoghurt drink	81.7	4.1

fats were used. Parameters of both methods were identical. Limits of detection estimated by blank samples analyses ranged from 5.2 to 14.7 mg/kg. Recovery 80–92%,  $C_v = 4.2$  and 6.8% for HPLC, 4.6 and 10.2% for GC determination. Linearity was  $R = 0.9997$  for all analytes in HPLC and GC method. Accuracy of the methods was proved on the standard reference material SRM 1845, Cholesterol in whole egg powder and was 95.7%.

Amounts of total cholesterol in various kinds of milk and milk products are stated in Tables 1 and 2. Content of stigmastrol and sitosterol in fresh butter were not detected, in butters with added vegetable fat were in accordance with producers statement.

## CONCLUSIONS

RP HPLC method for the determination of cholesterol and eventually of other sterols in milk and dairy products is a fast and reliable method alternative to the GC chromatography.

Parameters of both methods are comparable, and obtained results identical. If the matrix is not too complicated, further clean up procedures prior to the chromatographic determination are not

required. The sensitivity of the method (units of mg/kg) is for the determination of sterols in lipid fraction in milk products sufficient.

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