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Supercritical fluid extraction and chromatography of cholesterol in food samples

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ABSTRACT

A method based on supercritical fluid chromatography is presented which can be used for the determination of cholesterol in certain foods. The method involves the extraction with supercritical carbon dioxide and analysis of the extracts using a capillary column with supercritical carbon dioxide as mobile phase and flame ionization detection. Quantification is achieved using cholesteryl chloroacetate as an internal standard.

INTRODUCTION

In recent years, appplications of supercritical fluid chromatography (SFC) have been rapidly increasing[1,2]. Among the attributes of SFC are a high separation efficiency, appplicability to thermally labile compounds, compatibility with a wide range of detectors and flexibility in pressure, temperature and density control. Supercritical fluid extraction (SFE) has also attracted considerable interest as it is relatively easy to implement simultaneous control of volatility and solubility simply by programming the pressure and temperature or density and temperature simultaneously during an experimental run.

In both SFC and SFE, carbon dioxide is frequently employed as the mobile phase and as the extraction solvent, respectively, primarily owing to its inert properties, its compatibility with universal detectors (*e.g.*, the flame ionization detector) and its availability in high purity. In addition, carbon dioxide is supercritical at moderate temperature (*ca.* 31.1°C) and pressure (*ca.* 72.8 atm), thus making it a suitable choice from an instrumentation point of view. Further, SFE utilizing carbon dioxide is a solvent-free type of extraction where no toxic solvents are required and the carbon dioxide used can be easily removed by reducing the pressure.

The aim of this study was to investigate the applicability of SFC for the determination of cholesterol. Cholesterol was chosen as a test material because there are a number of inherent difficulties associated with its determination using conventional chromatographic techniques. Derivatization is often required to improve the detec-

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tion in the liquid chromatographic analysis of cholesterol[3]. Even though gas chromatography has been successfully carried out[4], a high temperature is required (*ca.* 260–300°C). Although cholesterol can be determined at lower temperatures by a number of other methods[5–9], these methods are either inaccurate or time consuming. Further, cholesterol is of great interest in both the food and medical sciences. It has been implicated in vascular pathology including coronary disease and there is therefore a need for a sensitive and accurate quantitative procedure for its determination[10].

In this paper, we report a simple technique based on SFC for the determination of cholesterol in food samples. The efficiency of a supercritical carbon dioxide ectraction method was compared with that of a Soxhlet extraction procedure employing *n*-hexane as the extraction solvent.

EXPERIMENTAL

The experiments were performed with a Model SFC 3000 system (Carlo Erba), equipped with a flame ionization detector. The column was a SE-52 fused-silica capillary column (10 m × 100 μm I.D., coating thickness 0.45 μm). Tapered restrictors rated nominally at 8 ml/min (J&W Scientific) were connected after the column for pressure reduction. Injections were made with an air-actuated Valco VICI injection valve equipped with a 1-μl loop. The injection time was 1 s. The chromatographic data were collected and analysed with a Hewlett-Packard Model 3390A integrator. The temperatures of the injection port, spliting outlet and detector were set at 40, 250 and 320°C, respectively. All runs were performed isothermally at 85°C. The pressure programming was from 14 to 20 MPa in 60 min.

All chemicals were of analytical-reagent grade or better. The standard and calibration solutions were prepared in HPLC-grade hexane (J. T. Baker). The standard solution contained 650 ppm each of cholesterol and the seven derivatives.

In order to select an appropriate internal standard, seven cholesteryl derivatives were analysed together with cholesterol in preliminary runs. The seven derivatives used were cholesteryl chloride, bromide, chloroacetate, *n*-hexanoate, *n*-heptanoate, caprylate and benzoate. The criteria for the choice of the internal standard were that it should not have a retention time too close to that of cholesterol and that it should elute witin a reasonably short time.

The design of the SFE system used in this study has been described elsewhere[11]. For the SFE of egg yolk samples, the pressure of carbon dioxide was kept constant at 17.7 MPa and the temperature at 45°C. An extraction time of 1 h was used. For the Soxhlet extraction procedure, *n*-hexane was used as the solvent and the extraction time was 7 h. In both instances the extraction times used were obtained by periodically analysing the extracts until no further improvement in the extraction efficiency could be obtained with further increases in the extraction time. For the calculation of the extraction efficiency of each type of extraction procedure, the recoveries of cholesterol and the internal standard from spiked glass-wool were determined. To illustrate further the applicability of the procedure to food samples, experiments were performed to extract cholesterol from egg yolk. The amount of egg yolk used for each extraction was 0.2 g and the amount of internal standard added to each sample was 0.03 g. The extracts were collected in a heated stainless-steel collector[11] and then dissolved in 100 ml of *n*-hexane. The solutions were evaporated to dryness under reduced pres-

sure. Subsequently, the dried extracts were dissolved in 4 ml of *n*-hexane for chromatographic analysis.

RESULTS AND DISCUSSION

Fig. 1 illustrates the chromatogram obtained for cholesterol and the seven cholesteryl derivatives. Based on the results, cholesteryl chloroacetate was chosen as the internal standard as it satisfied both of the selection criteria. The amounts injected were within the linearity range of the detector. The R^2 values of the linear fittings for cholesterol and cholesteryl were 0.9980 and 0.9995, respectively. Peak area reproducibility was within 2% relative standard deviation (R.S.D.) and retention reproducibility within 0.15% R.S.D. These values were obtained using manual injection in the splitless mode and with an identical flow restrictor. The detection limits for both cholesterol and cholesteryl chloroacetate were 25 ppm at a signal-to-noise ratio of 3.

In Table I the extraction efficiencies and levels of cholesterol in two egg yolk samples are given. The extraction efficiencies were obtained based on the recoveries of the internal standard in each sample. The levels of cholesterol shown were corrected for the efficiency of the respective extraction procedure and each value represents at least duplicate extractions. A typical chromatogram for an extracted egg yolk sample is illustrated in Fig. 2.

From the results in Table I, it can be seen that SFE is a more effective extraction method than the conventional Soxhlet procedure. The small difference in the amount of cholesterol determined were due to inhomogeneity of the sample, as two eggs were used. Although in both extraction methods recoveries as high as 98% could be achieved, SFE required only 1 h whereas Soxhlet extraction required 7 h. Compared with the extraction procedure employed by Hurst *et al.*[12], which involved hydrolysing the samples with potassium hydroxide, followed by a number of subsequent steps, SFE is much simpler and has been found to be capable of yielding superior extraction

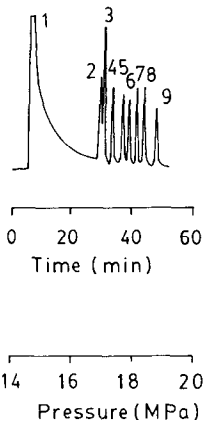

Fig. 1. Typical chromatogram for cholesterol and cholesteryl derivatives obtained under the following conditions: mobile phase, carbon dioxide; linear pressure programme from 14 to 20 MPa in 60 min; oven temperature, 85°C; injector temperature, 40°C; detector temperature, 320°C. Peaks: 1 = *n*-hexane; 2 = cholesterol; 3 = cholesteryl chloride; 4 = cholesteryl bromide; 5 = cholesteryl chloroacetate; 6 = cholesteryl *n*-hexanoate; 7 = cholesteryl *n*-heptanoate; 8 = cholesteryl caprylate; 9 = cholesteryl benzoate.

TABLE I

COMPARISON OF RESULTS OF EXTRACTION USING SFE AND SOXHLET EXTRACTION

Extraction method	*Efficiency (%)*	*Cholesterol in 100 g of egg yolk (mg)*
SFE	98.0	1447 ± 1.7
Soxhlet	98.0	1380 ± 2.0

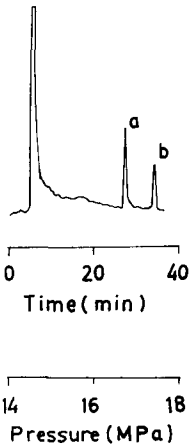

Fig. 2. Typical chromatogram of an extracted egg yolk sample. Chromatographic conditions as in Fig. 1. Peaks: a = cholesterol; b = cholesteryl chloroacetate.

efficiencies for cholesterol. Bearing in mind that the extraction selectivity and efficiency can be further improved by using pressure and temperature programming for the extraction of complicated samples, it is believed that it will be possible to develop the potential of SFE as a powerful alternative to many conventional extraction procedures.

CONCLUSION

The possibility of using low temperatures in SFC, as shown here, makes it a very attractive technique especially in the analysis of thermally labile compounds. Similarly in SFE the operating temperature required can be as low as 35°C for the extraction of cholesterol. This would mean that during the extraction procedure other thermally labile components in the food samples, such as protein, vitamins and various nutrients, would not be destroyed. For some applications where it is necessary to preserve the materials of the matrix for further analysis, SFE can be used advantageously. Further, it is worth noting that in the present procedure, minimum sample preparation is required. It is only necessary to dissolve the samples in an appropriate solvent. With the possibility of performing simultaneous pressure and temperature or density and temperature programming, there is ample flexibility in selecting the chro-

matographic and extraction conditions. It is therefore possible to develop a rapid and reliable method for the routine determination of cholesterol in biological samples using supercritical fluid technology.

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