FIRST STEPS OF DEVELOPMENT OF A METHOD FOR SIMULTANEOUS DETERMINATION OF CHOLESTEROL AND FATTY ACIDS IN FOOD MATRICES

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Received 08 January 2018
Accepted 20 April 2018

ABSTRACT

Cholesterol and fatty acids are compounds with a key role in the human organism. Their levels must be strictly controlled and maintained in the human body to ensure its proper biochemical functioning. Herein, we report first steps of development of a method for cholesterol determination in food using GC/FID. The calibration, the linearity and the choice of an appropriate internal standard are discussed. The obtained correlation coefficient is $R^2 = 0,9992$. A very good analytical yield of cholesterol 100 % is achieved following a specific procedure of sample preparation. LOQ is 50 ppm, while LOD is 15 ppm.

Keywords: cholesterol, GC/FID, fatty acids, betuline, α-methyltestosteron, squalen.

INTRODUCTION

Cholesterol (Fig. 1) is a sterol extremely important for the human body. Due to its presence the latter produces some vitamins (vitamin D), sex hormones (progesterone, testosterone, etc.), bile acid needed for digestion, stress hormones (cortisol). Cholesterol plays a key role as a neuro protector, in the cell membrane function, etc.

Cholesterol levels in the human body are extremely important because of its key role in many processes in organism biochemistry. According to the investigation of the cholesterol levels effect on human health, the total blood cholesterol levels, also named serum cholesterol level, has to be below 200mg/dL. If the level becomes greater than 240 mg/dL, there is an increasing risk of cardiovascular diseases, an ischemic heart attack, etc. [1]. 70% of the organism cholesterol is produced by the liver in correspondence with the human body needs like vitamins and hormones production. Another part, 30%, is introduced to the human body through nutrition. The possibility of monitoring the quantity of cholesterol coming from external sources to the body is of a great importance. Many groups investigate different methods of cholesterol analysis [2]. The most useful techniques include GC or HPLC [3, 4]. Some enzymatic methods for determination of cholesterol are also investigated [5]. Each of these methods has some particular characteristics concerning the type of the used standard as well as the specificity of the food matrix. The problem with cholesterol determination becomes more complicated if it has to be determined together with other contents of the matrix. Herein, we report the beginning of our investigation aiming to create a new method for simultaneous determination of cholesterol and fatty acids in food matrices by means of GC/FID technique.

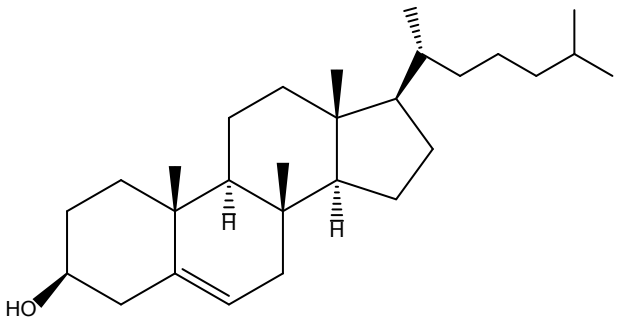

Fig. 1. Cholesterol structure.

EXPERIMENTAL

Materials, methods and apparatus

Betuline, α-methyltestosteron, squalane and iso-octane of an analytical grade were purchased by Sigma Aldrich.

Gas chromatograph Shimadzu QP 2010 equipped with autosampler and FID detector was used.

Procedure for GS/FID determination

Prior to calibration performance the gas chromatograph was adjusted according to the manufacturer's instructions and the operating conditions specified in the measurement method. First, a blank sample of pure solvent was injected to verify the system's functioning and to check the presence of a contamination. The identification of the aim compounds was performed by the retention time t_R (min). Several columns were studied aiming better separation: Supelco Omega Wax (15 m, 0.10 mm i.d., 0.10 um film thickness) for fast GC, Agilent J&W DB WAXetr (30 m, 0.32 mm i.d., 1.8 um film thickness) and Supelco SP-2380 (30 m, 0.25 mm i.d., 0.20 um film thickness). Supelco SP-2380 column was chosen because of the aim compound best separation and the retention time which was good for our purposes.

Chromatographic conditions

The parameters of methods referred to the starting temperature of 60°C for 2 min, the temperature gradient of 4°C/min to 250°C for 59.50 min, and the injector temperature of 260°C.

Standards preparation procedure

Standard solutions of cholesterol of concentrations of 50 ppm, 100 ppm, 200 ppm, 500 ppm and 1000 ppm in iso-octane were prepared in volumetric flasks.

Standard solutions in iso-octane of several possible internal standards like betuline, α-methyltestosteron and squalane were also prepared. Their concentration was equal to 2000 ppm.

Sample preparation for recovery determination

0,4 mL of the internal standard of a concentration of 2000 ppm was placed in a glass round flask. The solvent was evaporated under a nitrogen stream and 6 mL of 0,5M NaOH/CH_3OH was added. The obtained solution was refluxed for 10 min and 4mL of BF_3/CH_3OH was added. The resulted solution was refluxed for additional 5 min. 30 mL of saturated solution of NaCl were added to the hot solution. The obtained mixture was vigorously stirred for at least 30 s, cooled and 40 mL of distillated water were added. An organic layer was collected upon its separation. It was then dried on Na_2SO_4 (20-30 mg) and subjected to an analysis.

RESULTS AND DISCUSSION

In order to create a new method for analysis the following parameters have to be studied: Linearity; Working range of measurement; Analytical yield; Limits of Detection (LOD); Limits of Quantification (LOQ); Specificity/selectivity; Precision; Repeatability; Reproducibility; Uncertainty.

The first step of our work is to determine the linearity of the measurements. An interval between 50 ppm and 1000 ppm is chosen. The correlation coefficient is determined based on 5 samples for each cholesterol concentration studied. A very good correlation coefficient $R^2 = 0,9991$ (Fig. 2) is found.

The second step is to choose the appropriate internal standard for both fatty acid and cholesterol determina-

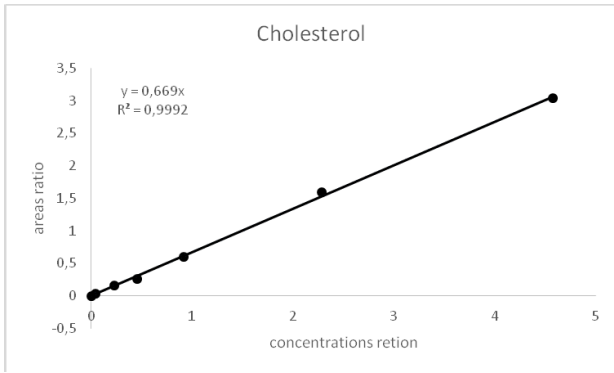

Fig. 2. A standard cholesterol curve.

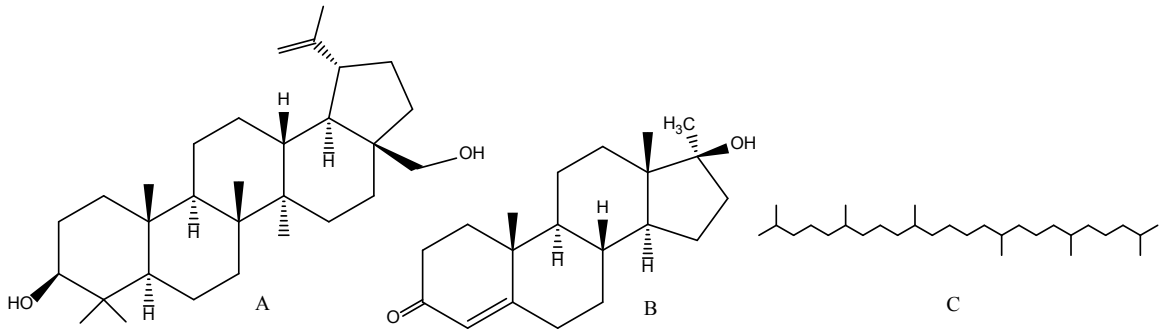

Fig. 3. Structures of the internal standards used: A. Betuline; B. α-methyltestosteron; C. Squalane.

tion. Several compounds are investigated: betuline, α-methyltestosteron and squalane (Fig. 3).

The first one is rejected because of its poor solubility in iso-octane chosen as the most appropriate solvent for extraction of both fatty acid and cholesterol. α-metyltestosterone has a very bad recovery following the saimple preparation procedure. Besides, the area of picks varies greatly for the same concentration (Fig.4 A and B).

Squalen is finally chosen as an internal standard because of the very good retention time obtained and its good recovery following the sample preparation procedure (Fig. 5).

The aim compound, cholesterol, and squalane as an internal standard are subjected to the procedure of sample preparation in order to determine the analytical yield. In our case, the assessment is carried out in food matrix absence. So, no interferences related to the food matrix can be observed. Three samples at three concentration levels of 50 ppm, 200 ppm and 1000 ppm are processed. The results referring to the analytical yields obtained are summarized in Table 1.

By definition LOQ is the smallest amount of the component determined, which can be calculated with a certain probability. If a technical instrument with a defined calibration band is used, the LOQ coincides with the first point in that range. For the specific case of cholesterol the value is 50 ppm.

Moreover, LOQ can be calculated using the following Eq. (1):

$$LOQ = 10S_0 \quad (1)$$

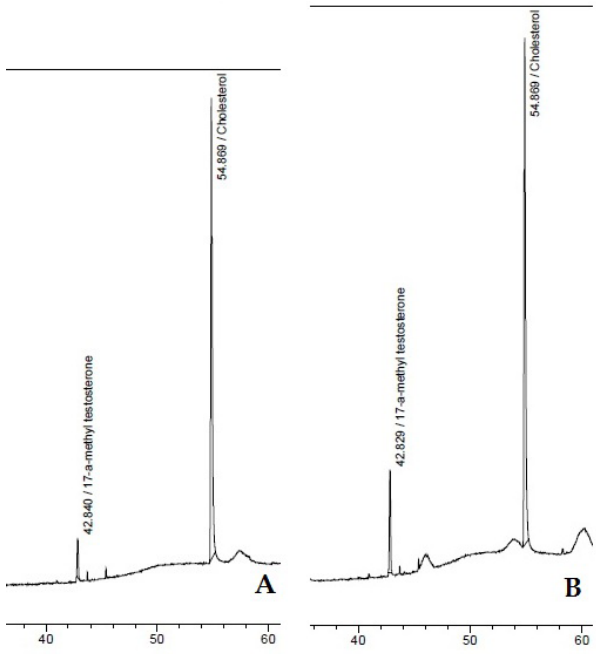

Fig. 4. Two different injections (A and B) of α-metyltestosterone at an identical concentration.

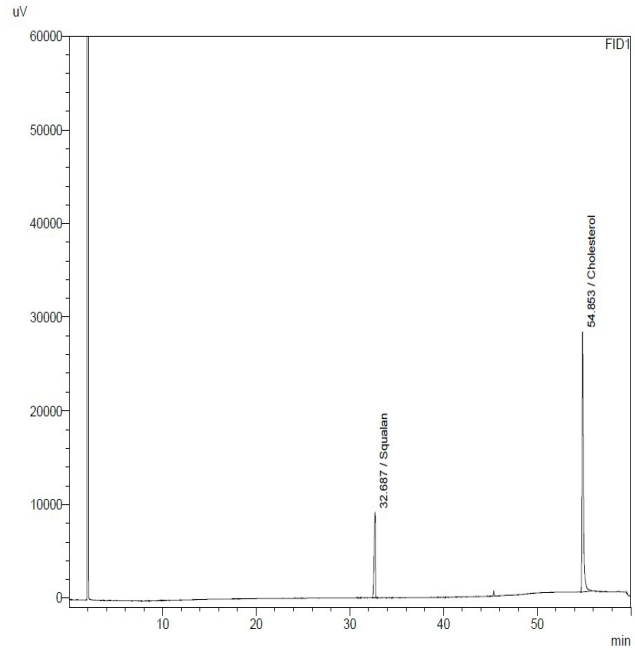

Fig. 5. Peaks separation observed in presence of cholesterol and squalane as an internal standard.

Table 1. Analytical yields of squalane as an internal standard and cholesterol at three concentration levels.

concentration [ppm]	analytical yield [%]	analytical yield [%]	analytical yield [%]
50	96,728	108,976	100,646
200	104,533	104,879	105,922
1000	102,855	104,168	106,969

Limit of quantification (LOQ)

where, S_0 is a standard deviation (SD) obtained using a test blank sample with a dimension equal to that of the measured quantity. The values of S_0 can be easily calculated and for the target compound S_0 = 5 ppm.

LOD represents the smallest amount of the component studied that can be detected with a certain probability. It can be calculated using Eq.(2):

$$LOD = 3S_0 \quad (2)$$

The calculated value of LOD referring to holesterol as a target compound is 15 ppm.

CONCLUSIONS

Squalane was determined as an appropriate internal standard for cholesterol determination. A very good correlation in the chosen interval between 50 ppm and 1000 ppm was obtained. The analytical yield for recovery of cholesterol was very good in the appropriate range between 70 % and 120 %.

Determination of the other parameters of the chromatographic method used as well as of the presence of both fatty acids and cholesterol in different food matrices is in progress.

Acknowledgements

This work is realized with the financial support of Science Investigation Center of UCTM through project 11688. Authors would also like to thank TC Globaltest for the opportunity to use GC/FID system.

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