METHOD VALIDATION OF CHOLESTEROL ANALYSIS USING HPLC - ELSD ON EGG AS SAMPLE MATRIX

Tika Setianingrum and Hanifah Nuryani Lioe
Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, IPB Darmaga Campus, PO BOX 220, Bogor, West Java, Indonesia
Phone: +62 813 15830421, E-mail: tika_sn@yahoo.com

ABSTRACT

A high-performance liquid chromatography (HPLC) method for the separation and quantification of cholesterol using an evaporative light-scattering detector (ELSD) is described. Detection of cholesterol was achieved in 1.50 until 1.53 minutes using cholesterol standard. A simplified binary mobile phase mixture of hexane and isopropanol (90:10) was used to separate cholesterol. Detection was accomplished with an ELSD, with the following settings: evaporation temperature 50 °C, air pressure 2.2 bars, flow rate of mobile phase 2 mL/min and grain 7. A calibration curve was obtained from 0 to 5000 µg/g sample, the linear equation of cholesterol is \( y = 35.58x - 3140 \), with a correlation coefficient of 0.997. Instrument detection limit (IDL) was 1.07 µg/mL and limit of quantitation (LOQ) was 3.56 µg/mL. Recovery by spiking cholesterol standard in egg sample at low concentration was 122.13%, medium concentration was 108.23%, and high concentration was 44.71%. Method detection limit (MDL) value was 2.30 µg/g sample. With intra reproducibility value was 0.04%.

Keywords: HPLC-ELSD, validation, cholesterol, eggs