Abstract:

Phospholipids are an important group of polar lipids constituting the main component of cell membranes. Their proportion may vary depending on many factors of the surrounding environment in which the cell is located. Determination of membrane phospholipids is essential in many scientific, industrial and economic sectors.

The aim of this work has been to develop a separation method for determination of membrane phospholipids by which it was possible to analyze phosphorylated parts of phospholipids and fatty acids from one sample. Comprehensive gas chromatography with mass detection (GC×GC-MS) was chosen for the assay. This method allows the separation of the entire sample on two serially connected different columns, among which is the interface called modulator.

The preparation of the sample includes a cleavage of the phospholipid molecule by the enzyme phospholipase C, which released the phosphorylated polar headgroups. These polar parts had to be derivatized before analysis. The principle of the chosen derivatization consisted in the use of two different silylation agents (hexamethyldisilazane and *N,O*-Bis(trimethylsilyl) trifluoracetamide) in two steps. Conditions were selected for efficient separation of the silylderivatives of phosphorylated headgroups using GC×GC-MS using a cryogenic modulator. Individual silylderivatives were identified by mass spectra from the MS detector and their characteristic intensive fragments were selected.

The developed method was tested on real samples of membrane lipids of *Bacillus subtilis*. Silylderivatives of phosphoric acid, phosphoglycerol, phosphoethanolamine and phosphoserine were identified in the samples.

Keywords:

phospholipids, enzymatic hydrolysis, fatty acids, derivatization, comprehensive gas chromatography, biological samples