Abstract

Eicosanoids represent large group of biologically active lipid metabolites syntesized from polyunsaturated fatty acids that play an important role in many physiological processes. To describe complex metabolism of these compounds, analytical methods including extraction from a biological sample using solid phase extraction and liquid chromatography coupled with mass spectrometer detection were used.

Solid phase extraction of biological samples was optimized on four types of reverse phase columns of which column Strata X 60 mg/3 ml, 33 um (Phenomenex , USA) was the most effective. Also, alternative solid phase extraction of eicosanoids using columns with ion exchange sorbents and a column with normal phase were tested, but proved to be unsuitable for targeted analysis of eicosanoids. The extraction method yielding the best results - Strata X 60 mg/3 ml, 33 μm (Phenomenex, USA) was used for the separation of eicosanoids from mouse gonadal fat samples. Eicosanoids were analyzed by liquid chromatography and the separation mechanisms were tested on three UPLC core-shell columns of different lengths (50 mm, 100 mm, 150 mm). The most effective separation of prostaglandin E2 and prostaglandin D2 was achieved using the longest column Kinetex 150 mm × 2,1 mm, 2,6 μm. Furthermore, ionization parameters, such as declustering potential, of selected eicosanoids were optimized and fragmentation of prostaglandin D2, including modulation of collision energy, were explored in detail. Final method optimized for the analysis of eicosanoids was used to measure quantity of selected eicosanoids in gonadal fat, liver and heart of mice.