ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Organic and Bioorganic Chemistry

Candidate: Seyedmohammad Khatibi

Supervisor: PharmDr. Lukáš Opálka, Ph.D.

Consultant: RNDr. Martina Navrátilová, Ph.D.

Title of diploma thesis: Optimization of the method for quantification of lipids in human corneocyte lipid envelope

Ceramides are essential sphingolipids in the skin, playing crucial roles in barrier function and cellular regulation. This study aimed to optimize the isolation of covalently bound ceramides from the skin's *stratum corneum* by developing and refining extraction and saponification procedures. Specifically, the focus was on ceramide types NS (free lipids), OS, OH, OdS, and OP, obtained from samples of three healthy individuals.

Eight layers of the *stratum corneum* were collected from each participant and merged to form a composite sample. The isolation procedures were divided into three main steps: extraction of free lipids, purification of the remaining pellet, and saponification of covalently bound lipids. The procedures were systematically tested under varying conditions across seven experiments to determine the optimal parameters for ceramide extraction.

Initial experiments revealed that high temperatures during extraction and saponification led to significant degradation of covalently bound lipids. The first experiment, using high-temperature procedures, resulted in the complete destruction of covalently bound lipids. Subsequent experiments demonstrated that lower temperatures improved the stability of ceramides. In particular, with modified conditions including a bath temperature of 40°C for purification and 37°C for saponification, several ceramide types were successfully isolated. This approach revealed significant quantities of ceramide OS, ceramide OH, ceramide OdS, and ceramide OP. Ceramide NS was found in minor quantities, indicating the effective removal of free lipids.

Further refinement of conditions, which varied in saponification temperatures and times, showed that increased saponification time enhanced the extraction of OS and OdS ceramides. Specifically, longer saponification times improved the recovery of OS ceramides, while high temperatures adversely affected the stability of free lipids and some ceramides.

This study identified the critical role of temperature and washing/saponification times in optimizing ceramide extraction. Lower temperatures and extended washing and saponification times significantly improved the recovery of ceramide types OS, OdS, and OP, while high temperatures led to degradation. The optimized procedures outlined in this study provide a reliable method for isolating and analyzing ceramides, offering insights into their distribution and potential implications for skin health research.

Keywords: Ceramides, Sphingolipids, *Stratum corneum*, Lipid envelope, Covalently bound lipids, Lipid extraction, Saponification, Skin barrier, Lipid quantification, Temperature effects on lipids, Skin health research.