

ABSTRACT

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Title of diploma thesis: Sequential injection chromatography - testing and comparison of modern chromatographic columns

This diploma thesis deals with testing and comparison of two modern chromatographic columns in Sequential injection chromatography system. They were used for separation of selected vitamins B (thiamine, nikotinamide, pyridoxine, riboflavin and folic acid) and vitamin C. Developed method was based on UV detection at 220, 272 and 290nm. The Ascentis Express HILIC column (3 cm x 4.6 mm, 2.7 μ m) was tested by using acetonitrile with ammonium acetate as a mobile phase. The method was not successfully developed because of low retention times of vitamins. The separation of six vitamins was not able, because of low selectivity. There was developed a method with use of Ascentis Express RP-Amide column (3 cm x 4.6 mm, 2.7 μ m) using gradient elution, which was able to separate all 6 vitamins. Acetonitrile and phosphoric acid (pH 1.8) were components of the mobile phases. There was made calibration, reproducibility and recovery tests, but there was not good results enough to validate the method. For recovery was used energetic drink SHOCK! containing these vitamins.