Abstract

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Title of the diploma thesis: Sequential injection chromatography - advanced and two-dimensional separations

The subject of this work was the development of two analytical methods intended for separating substances contained in silymarin, their comparison and preparing a template in the Microsoft Excel program to simplify data evaluation.

In the first developed method, called classical sequential injection chromatography (1D-SIC), a long Ascentis® Express F5 chromatography column (10 cm x 4.6 mm, 5 μm) with an opti-guard® C18 pre-column (1 mm) was used during analysis. The mobile phase consisted of a mixture of acetonitrile with phosphoric acid solution with pH 2.0 prepared in a ratio of 27:73. The isocratic mode was chosen for the analysis; the total length was 16.3 minutes with a flow rate of 5 μL/s.

In the second developed method, called two-dimensional sequential injection chromatography (2D-SIC), a Kinetex® 5 μm EVO C18 column (30 x 3.0 mm) with an opti-guard® C18 pre-column (1 mm) was chosen for the first dimension. In the second dimension, an Ascentis® Express F5 column (30 mm x 4.6 mm, 2.7 μm) was used. The analysis mode was chosen isocratic with a total length of 35.3 minutes with a flow rate of 10 μL/s. A mixture of acetonitrile with phosphoric acid solution with pH 2.0 prepared in a ratio of 30:70 was used as the mobile phase.

During each analysis, the sequential injection chromatography system was controlled by SIAsoft v1.1.7 computer software. A detailed description of the system and program settings for individual analyses is contained in the text of this work.

A complex template was created in the Microsoft Excel program, which significantly facilitated the evaluation of the obtained data during separations.

Keywords: sequential injection chromatography, 1D-SIC, 2D-SIC, silymarin