Abstract

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Name of the thesis: Comparsion of columns for separation of pharmaceutically significant substances by method of Sequetial Injection Chromatography

This diploma thesis deals with the development of the separation method in the system of the Sequential Injection Chromatography (SIC). The used SIC system was based on the system SIChrom™ (FIAlab®, Bellewue, WA, USA) with the eight port stainless steel multi-position valve (VICI® Valco Instruments, TX, USA) and with the pump S17 (Sapphire™ Engeneering, MA, USA).

The samples were prepared by dissolving of 25 mg of various phenolic acids (protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, sinapinic acid and syringic acid) in 25 ml of methanol.

Three fused-core particle columns were used in this thesis : the column Ascentis Express C-18 (30 mm × 4.6 mm, 2.7 µm, Sigma – Aldrich, Supelco Analytical, Bellefonte, PA, USA), the column Ascentis Express RP-Amide (30 mm × 4.6 mm, 2.7 µm, Sigma – Aldrich, Supelco Analytical, Bellefonte, PA, USA) and the column Ascentis Express Phenyl-Hexyl (30 mm × 4.6 mm, 2.7 µm, Sigma – Aldrich, Supelco Analytical, Bellefonte, PA, USA).

The mobile phase was prepared by mixing water and acetonitrile in different ratios. Its pH was adjusted by the 85% phosphoric acid to the value of 2,40.

The conditions for the column C18 are as followed: the mobile phase 16:84 (acetonitrile:water v/v; pH 2.36), the volume of the mobile phase 3 600 µl, the flow velocity 10 µl/s and the volume of the sample 10 µl.

The conditions for the column RP-Amide are as followed: the mobile phase 22:78 (acetonitrile:water, v/v; pH 2.40), the volume of the mobile phase 3 800 µl, the flow velocity 6 µl/s and the volume of the sample 10 µl.

The conditions for the column Phenyl-Hexyl are as followed: the mobile phase 16:84 (acetonitrile:water, v/v; pH 2.46), the volume of the mobile phase 3800 µl, the flow velocity 10 µl/s and the volume of the sample 10 µl.

Analyses were isocratic.

The absorbance was monitored at 250 nm (vanillic and protocatechuic acids), 280 nm (syringic and *o*-coumaric acids) and 290/325 nm (sinapinic, ferulic and *p*-coumaric acids). The used UV source was the lamp UV D-1 000-CE (Analytical Instrument Systems Inc., USA) and the used detector was the detector CCD UV-VIS USB 4 000 (Ocean-optics, FL, USA).

The used flow cell was the Z-cell Ultem® SMA, effective length 20 mm (FIAlab®, Bellewue, WA, USA).

The separation method was developed for each column and the basic separation features of the columns were compared to each other.

The column RP-Amide was the best one in the selectivity because it separed all seven acids. The columns C18 and RP-Amide were not able to separe two pairs of acids. The two peaks were the double peaks and it was found out that the double peaks belonged to vanillic and syringic acids and ferulic and sinapinic acids.

The column C18 was the best one in the calibration because its calibration curves approach very much to the straight line.

The column C18 exhibits the lowest relative standard deviation (RSD) for the retention time and the response in the field of the high concentrations.

The column RP-Amide has the lowest RSD of the response for the middle concentrations and the lowest RSD of the retention time for the low concentrations.

The column Phenyl-Hexyl shows the lowest RSD of the retention time for the middle concentrations and the lowest RSD of the response for the low concentrations.

The column RP-Amide reports the most symmetrical peaks for the all concentrations and it has the highest effectiveness. The highest values of the peak resolution were measured by the column RP-Amide, too.

The separation method was developed for all three columns successfully.

The column RP-Amide is the best column for the analysis in this diploma thesis.