ABSTRACT

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Bc. Olga Válová
Supervisor: Doc. RNDr. Dalibor Šatínský, Ph.D.

Title of diploma thesis: Two Dimensional Separation in the Low Pressure System of Sequential Injection Chromatography

The subject of this work was to construct a new method for separation of two analytes, which are different in their chemical properties and lipophilicity.

Newly described method named Two Dimensional Sequential Injection Chromatography (2D-SIC) for isocratic system was applied in this work for the analysis of pharmaceutical preparation Otobacid N, which contains active substances Dexamethasone and Cinchocaine, i.e. substances with different chemical properties.

The separation was carried out in two dimensions of two columns. In the first dimension for separation of Dexamethasone was used column 25 x 4.6 mm C-18 monolithic column with mobile phase acetonitrile/water in ratio of 35/65, flow rate 15 μl/sec. In the second dimension for separation of Cinchocaine was used column 10 x 4.6 mm C-18 monolithic column with precolumn 5 x 4.6 mm C-18 with mobile phase acetonitrile/water in ratio 60/40, flow rate 15 μl/sec. Optimal wavelength at 240 nm was chosen by spectrophotometric detection.

Relative standard deviation (RSD) of repeatability ranged from 1.88 % to 3.10 % for Dexamethasone and from 0.37 % to 2.26 % for Cinchocaine. RSD of precision was 3.12 % for Dexamethasone and 1.72 % for Cinchocaine. And RSD of recovery for Dexamethasone: 2.63 % and Cinchocaine: 1.95 %. The percentage of recovery was 99.02 % for dexamethason and 99.48 % for cinchocain.

The correlation coefficient of linearity was determined by the value of r^2=0,99911 for Dexamethasone and r^2=0,99969 for Cinchocaine.

Two Dimensional Sequential Injection Chromatography was compared with HPLC method under isocratic conditions of separation and with used monolithic column 25 x 4.6 mm C-18.

Keywords: Flow Injection Analysis (FIA), Sequential Injection Analysis (SIA), Sequential Injection Chromatography (SIC), Two Dimensional Sequentioal Injection Chromatography (2D-SIC), dexamethasone, cinchocaine