

Abstract

The Thesis combines a theoretical part focused on better understanding of separation processes and interaction mechanisms with experimental applications for specific analytical purposes.

HPLC is one of the preferently used separation techniques. As various interaction types can participate in the retention mechanism of analytes, the choice of the optimal separation system is often ambiguous. The selection of suitable separation conditions is very often made empirically. Such approach is rather time consuming. Therefore, it could be advantageous to utilize some physicochemical characteristics of the separation system. Linear free energy relationship (LFER) is one of the comprehensive semiempirical methods that allows characterization and comparison of stationary phases/separation systems and yield to better understanding of the intermolecular interactions, which play role in the separation processes. The optimization of separation processes can be disturbed or complicated by phenomena called system peaks. In the case of detectable system zone the resultant chromatogram includes more peaks than is the number of analytes in the sample. A serious problem that can arise if an analyte interacts with the system zones is resonance. This phenomenon can disturb completely the separation results and make evaluation of the measured data impossible.

The LFER method was applied for characterization of interaction possibilities of three teicoplanin-based chiral stationary phases (namely teicoplanin, teicoplanin aglycone, methylated teicoplanin aglycone) under various reversed-phase (RP) conditions.

Behavior of system peaks was studied in RP HPLC systems consisting of an RP Amide C16 column and aqueous solutions of organic acids with alkaline metal hydroxides as mobile phases without any organic modifier. The ternary mobile phase used contained LiOH, benzoic acid, and tropic acid in different concentrations. Binary mobile phases yielded two system peaks; ternary mobile phases resulted in three system peaks after injection of a disturbance of LiOH. The first system peak was stationary while the second or second and third moved with changes of concentration of the buffer components.

RP HPLC methods for separation of selected estrogens and 1,4-benzodiazepines were developed. The method for enantiomeric purity control of the veterinary drug (\pm)-cloprostenol was optimized and validated.