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BASIC PRINICPAL OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Violeta Ivanova-Petropulos[1]

e-mail: violeta.ivanova@ugd.edu.mk

[1] Faculty of Agriculture, University "Goce Delčev" - Štip, Krste Misirkov bb, 2000 Štip, Republic of Macedonia

High-performance liquid chromatography (HPLC) is an analytical technique based on the separation of molecules due to differences in their structure and/or composition. Separation is performed between two phases, mobile and stationary. The molecules in the sample have different affinities and interactions with the stationary support, leading to separation of molecules. Compounds which are longer retained at the stationary phase will elute later, compared to those which are distributed into the mobile phase. Elution of the molecules can be achieved by isocratic or gradient method. In the isocratic method, the composition of the mobile phase remains constant, whereas in the gradient method the composition changes during the separation process. The complexity of the analytical problems requires confirmation of the identity of the peaks through additional qualitative information. This can be achieved by several types of detectors, such as: diode array detector (DAD), fluorescence detector (FLD), refractive index detector (RID) and mass spectrometer (MS). In general, HPLC is used for separation and quantification of polar and non-volatile components.

Key words: HPLC, elution, detection, quantification.