Abstract

This work brings extending of applications of sequential injection analysis in area of pharmaceutical analysis. It is focused on integration of monolithic columns into SIA system that enables simultaneous determination of few analytes in samples with matrix (biologic samples) or simple mixtures (pharmaceutical preparations). A new created method was called Sequential Injection Chromatography (SIC) and represents a new generation of flow methods.

Integration of short monolithic column with low back-pressure leads into creation of low pressure flow separation method. Commercially produced monolithic columns with reverse phase sorbent C18 are suitable for determination of active compounds and adjuvants in pharmaceutical preparations. This enables fast determination of samples without or only with simple off-line pre-treatment of sample. Employment of various lengths of monolithic columns used in SIC was studied.

First experimental work (appendix I) was first SIC experiment. The aim of it was trouble-free connection of monolithic column with SIA manifold and application on determination of pharmaceutical preparation Diclofenac HBF emulgel (ointment contains diclofenac nitrate, methylparaben and propylparaben). All parts of system were optimized for higher work pressure. Sample of ointment was pre-treated (extracted into methanol) before analysis. Gradient of flow speed of mobile phase was used for decreasing of time of whole analysis. Comparison of results with HPLC showed that SIC is good alternative method for determination of simple mixtures.

Second experimental work (appendix II) was focused on developing of simple and fast method for determination of two analytes in Sanorin® 0.5‰ (nasal and eye drops - contain naphazoline nitrate and methylparaben). Experience from previous works was used so that mainly pre-treatment of sample, mobile phase and internal standard were optimized. Only dilution with mobile of drops was done before measurement because adjuvants did not interfere. Sensitivity of determination was increased by use of two wavelengths for measuring in absorption maximum of both analytes. Results were compared with HPLC results under same chromatographic conditions.

Third experimental work (appendix III) deals about developing of method for simultaneous determination of betametasone and chloramphenicol in eye drops Betabioptal. Development was focused except chromatographic conditions on pre-treatment of sample – suspension eye drops. Results of validation were compared with method developed in HPLC

(different chromatographic conditions) and were gather suitable for routine analysis of all active compounds of pharmaceutical preparation.

The aim of fourth experimental work (appendix IV) was to develop SIC method with Onyx™ Monolithic C18 column. Both active substances of Triamcinolon-IVAX (topical solution - contains triamcinolon acetonide and salicylic acid) were determined. Pre-treatment of sample before analysis was very fast (only diluting with mobile phase) because adjuvants did not interfere. Concentration of triamcinolon acetonide in sample was ten time less than concentration of salicylic acid so only one wavelength (absorbance of triamcinolon acetonide) was used for adequate response. Results were compared with HPLC results under same chromatographic conditions.

Review work (appendix V) is an overview of all SIC works created in Department of analytical chemistry and comparison with HPLC. Advantages and disadvantages of SIC method were critically evaluated. Recent trends in flow methods show that separation in low pressure flow methods is hopefully developing area of analytical chemistry.

Number of published papers works show SIC like good alternative of HPLC for determination of simple mixtures (contain 2 – 5 analytes) ideal for drug control, drug efficiency and long time stability studies.