Abstract

This thesis is dedicated to the development of a method for the determination of 5-fluorouracil by high-performance liquid chromatography, which could be used for determination of its encapsulation efficiency by liposomes.

First, separation of 1 mM standard of 5-fluorouracil was tested on several types of columns. Among tested columns belonged a C18 column, a C18 column with positive surface modification, a phenyl-hexyl column and several fluoride columns together with two HILIC columns. A mixture containing 10 mM CH_3COONH_4, pH = 4,5 and MeOH in the ratio of 98/2 was initially used as the mobile phase.

The results showed, that 5-fluorouracil elutes on all columns too close to the dead time, where different impurities often elute. To avoid possible distortion of 5-fluorouracil signal by impurities potentially occurring in the real sample, several adjustments of chromatographic conditions were tested.

The most effective solution was addition of 5 mM ion-pairing agent (namely tetrabutylammonium chloride hydrate) to the mobile phase, together with adjusting pH to 8,0 to support the ionization of the analyte. In the combination with mobile phase modified in this way, a phenyl-hexyl column with retention time 4,36 minutes reached the best result, therefore it was selected for the final determination of 5-fluorouracil in real liposome samples.

The optimized method for the determination of 5-fluorouracil used Poroshell 120 Phenyl-hexyl column (100 × 2,1 mm; 2,7 µm; 120 Å) and a mobile phase of 10 mM CH_3COONH_4, pH = 8,0 + 5 mM tetrabutylammonium chloride hydrate and MeOH in a ratio of 98/2. The limit of detection of this adjusted method was 4 $\mu mol \cdot l^{-1}$ and limit of quantification 14 $\mu mol \cdot l^{-1}$.

This optimized method was tested in practice by determining free 5-fluoruracil in samples of four types of liposomes, by which encapsulation efficiency for the analyte was calculated based on the observed concentration.

Key words

high-performance chromatography, ion-pair chromatography, liposomes, 5-fluorouracil, UV-VIS detection