Laboratory exercise to advanced practice of analytical chemistry – JS 2007/2008 Supervisor Ing. Blanka Hégrová

High-performance liquid chromatography – the input for synthesis oligonucleotides, purity check

THEME GOALS – verify characteristics of liquid chromatography in exercise.

Conditions:

- basic knowledge of theory behind HPLC (written test)
- knowledge of the theme (written test)
- laboratory coat, appropriate foot-wear
- eventual lunch one after one ;-)

Definition of the analytical problem

Nowadays, oligonucleotides synthesis is a common part of routine molecular biology. With this we used single-purpose devices, synthesizers, which from original substances, herein nucleotides, synthesized required strings.

The pure input is basic requirement for high-quality synthesis. Decrease of yield can be caused by ballasts reactive in the same way. Therefore it's important at the first to find out the input quality. In regard of chemical impurities character (isomers) is one possibility of separation by using HPLC:

The suitable input: isomers amount < 3 %

The unsuitable input: isomers amount > 3 %

Definition of theme

Determination of percentage ballasts amount in guanosine-5´-monophosphate sample by HPLC.

Samples

Standards of nucleotide's bases: guanosine-5'-monophosphate (5-GMP),

guanosine-2'-monophosphate (2-GMP),

guanosine-3'-monophosphate (3-GMP).

Library of UV-VIS spectra of purines, pyrimidines, nucleosides and nucleotides.

Guanosine-5'-monophosphate sample.

Mobile phase: 25 mM phosphate buffer, pH 7.0.

Apparatus

HPLC system **10 AVP** by SHIMADZU (degasser GT-154, system control unit SCL-10AVP, 2x pump LC-10AVP, oven CTO-10ASVP, PDA detector SPD-M10AVP, control software Class-VP 5.02), colony 2x100x4.6 mm, Onyx C18, monolithic, particle size: 13 nm mesopores, $2 \mu m$ macropores.

OPERATING SEQUENCE

Analysis of sample 5-GMP

1. Chromatographic analysis of solution sample input 5-GMP.

Analysis of standards

- 1. Chromatographic analysis of 5-GMP standard solution.
- **2.** Chromatographic analysis of 2-GMP standard solution.
- 3. Chromatographic analysis of 3-GMP standard solution.

Identification of components, quantification and ballast amount determination

- **1.** Identification of individual sample components using retention times and UV-Vis spectra library.
 - 2. Using chosen calibration method, determination of percentage ballasts amount.
 - 3. Based on obtained data, determination of suitability synthesis input.
 - **4.** Comments to results as to analytical approach.