ABSTRAKT

Diploma thesis

The purification of bioconjugates of azaphthalocyanines by SPE

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This thesis occupy the development of a method for purification biocunjugate azaphthalocyanines by solid-phase extraction (SPE). These derivates of azaphthalocyanine are investigated as molecular probes to guench of fluorescence in various genetic analysis.

Experiments were carried out successively in order to find the ideal SPE conditions for the analyte. We tried also different type of SPE columns and their influence on the extraction. Subsequently were optimized each extraction steps, the changes in pH, molarity and strength elution solutions were made with an effort to get the best separation results. Column appeared as the best DSC-Ph (500 mg/3 ml) and the most optimal conditions were:

■ Condition: 3 ml 100% MeOH + 5 ml 50mM TEAA

■ Apply sample: 100 µl 100nM

Washing solution: 9 ml 55% MeOH/50mM TEAA
Elution solution: 3 ml 80% MeOH/50mM TEAA

We obtained by this method recovery of extraction analyte around 70-75 %, with 10% relative contamination of remains oligonucleotide chains in the final eluate solution. The SPE method is an alternative to the already developed HPLC method for purification bioconjugates of azafthalocyanines.

Keywords: Bioconjugates of azaphthalocyanines, SPE, sample preparation, HPLC.