ABSTRACT

Diploma thesis

The employment of HPLC for separation of bioconjugates of azaphtalocyanines

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This diploma thesis deals with development of suitable chromatographic conditions for separation of bio-conjugates of azaphthalocyanines. These compounds are of great interest today, they might be useful as potential quenchers of fluorescence in molecular probes.

The experiments were carried out in analytical and semi-preparative scale. Nine C18 chromatographic columns were tested. The conditions for analytical and semi-preparative chromatography were optimized.

The following chromatographic conditions for separation of bio-conjugates of azaphtalocyanines were found (analytical method):

- Stationary phase: Hypersil BDS, 100 × 4.6 mm, 3 μm particle size
- Mobile phase A: 50 mM water solution TEAA in 12 % acetonitrile;
- Mobile phase B: acetonitrile;
- Gradient elution.
- Other conditions: column temperature 40 °C, flow rate 1 ml per minute
- Detection 254 nm, 374 nm, 552 nm, 673 nm
The following chromatographic conditions for separation of bio-conjugates of azaphtalocyanines were found (semi-preparative method):

- Stationary phase: Hypersil BDS 250 × 4.6 mm, 5 μm particle size
- Mobile phase A: 50 mM water solution TEAA in 12 % acetonitrile;
- Mobile phase B: acetonitrile;
- Isocratic elution
- Other conditions: temperature 40 °C, flow rate 1 ml per minute
- Detection: 254 nm, 374 nm, 552 nm, 673 nm