

Isolation Of Oligonucleotides By Scalable IEX-Centrifugal Partition Chromatography

There is a growing need for simple, rapid and if necessary, easily scalable chromatographic purification of oligonucleotides. The use of ion-exchange (IEX) chromatographic techniques has increasing potential, moreover, liquid-liquid chromatography can be used to develop easily scalable and cost-effective solutions without the need for expensive ion-exchange resins. In this study, we demonstrate the potential of our centrifugal partition chromatographic solutions for the separation of oligonucleotides.

The Technology

Centrifugal partition chromatography (CPC) is a preparative liquid-liquid chromatographic technique, where both the stationary and the mobile phase are liquids and resolution is governed by the partitioning of solutes between these two immiscible liquid phases. In practice, one of the two phases is immobilized by a strong centrifugal force inside a rotor, while the other one, which contains the sample to be purified, is pumped through the rotor.

CPC is a very loadable system depending on the amount of exchanger and displacer salts, however for smaller sample quantities (100-200 mg) the concentration of additives can be reduced, thus further reducing costs.

A challenge in oligonucleotide separation is the presence of endotoxins, which are difficult to remove. The solvent system used in CPC can separate endotoxins, inasmuch as *n*-butanol/water extraction of endotoxins is a potential solution.

Case Study

The starting material was a 20-mer single-stranded unmodified oligonucleotide (dT), with 88.5% purity (**Fig. 1.**). For the separation, our two-phase solvent system consisted of a mixture of ethyl acetate/*n*-butanol/nuclease free water with a defined composition. The cationic compound (as exchanger molecule) was dissolved in the stationary phase responsible for the retention of oligonucleotides, while sodium hydroxide and sodium chloride dissolved in the mobile phase were responsible for the elution. The 600 mg sample was dissolved in 10 ml mobile phase and injected via injection loop. The applied flow rate was 10 ml/min.

Fig. 2. shows the purity and calculated yield of the collected fractions:

- fractions 36-51 achieved 96.6 purity and 94.5% calculated yield,
- fractions 38-49 achieved 98% purity with 74.5% calculated yield.

To remove the organic solvents, fractions were lyophilized, and salts and residual additives were easily removed by alcohol precipitation method.

In the purification of the oligo dT sequence used as the experimental material, 600 mg of sample was successfully purified to 96.6% purity at 94.5% calculated yield (**Fig.3.**). CPC can produce a high purity, high yield product without the use of expensive solid stationary phase, in a single purification step.

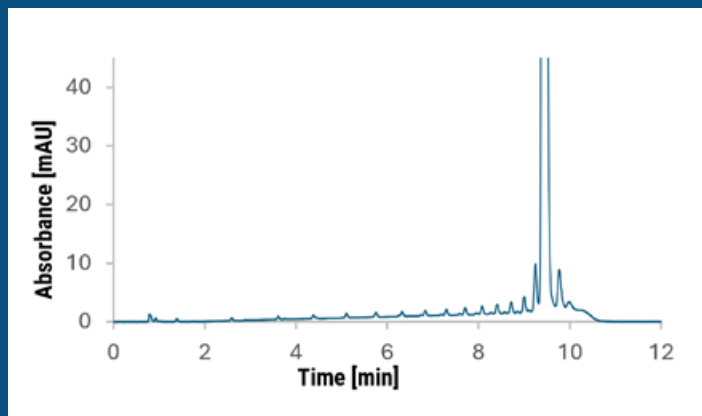


Fig. 1. AEX-HPLC analytical chromatogram of the crude sample.

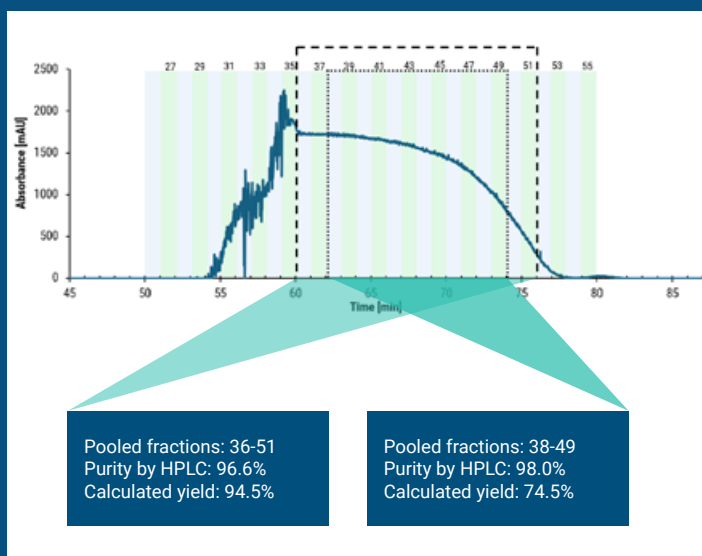


Fig. 2. Chromatogram of the CPC separation. The collected fractions (coloured stripes & numbers and pooled fractions are marked.)

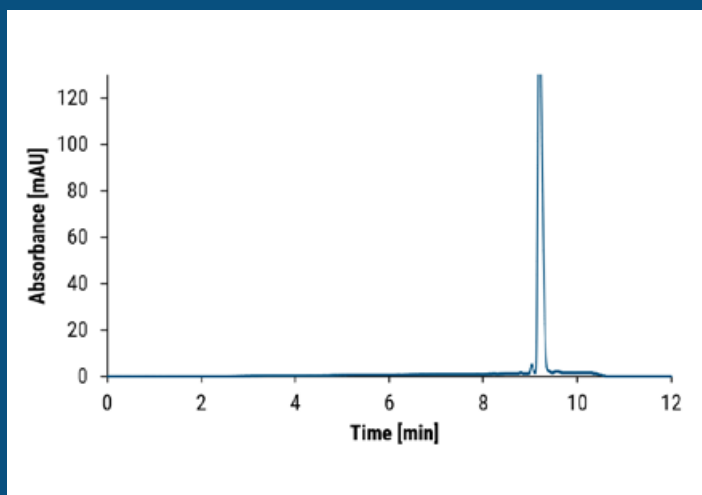


Fig. 3. AEX-HPLC analytical chromatogram of fraction 42.

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