Quality control of therapeutic oligonucleotides using HILIC-UV and CZE-UV

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1. Introduction

Oligonucleotides are short DNA or RNA fragments, usually composed with 5 to 30 nucleotides. In some cases, the sequence contains some chemical modifications such as a phosphorothioate (PS) link instead of a classical phosphodiester link between nucleotides. This modification is one of the most used in drugs formulation due to its several advantages towards the pharmacokinetic parameters namely more resistance towards degradation, decrease of their renal elimination, etc. On the analytical point on view, the addition of PS links brings more challenge to the separation due to the generation of diastereoisomers (2n-1 configurations with n = length of the oligonucleotide).

Synthetic oligonucleotides are booming therapeutic medicines as some drugs have recently been launched on the market. Additionally, an increasing number of molecules are currently in clinical trial. This is due to the interesting ability of oligonucleotides to specifically target biomolecules such as DNA, RNA, proteins etc. Therefore, reliable analytic techniques have to be developed in order to guarantee the quality of these medicines. The use of HILIC-UV and CZE-UV for the analysis of synthetic oligonucleotides were both investigated in terms of separation and resolution.

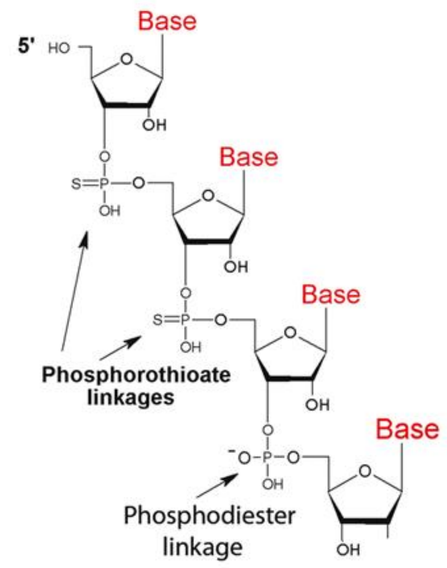

Fig. 1 : Oligonucleotide structure (Base = adenine, thymidine, cytosine, guanine for DNA & uridine for RNA)

2. Results

Three samples containing different mix of oligonucleotides were tested with both HILIC and CZE:

Samples	Peaks (Fig.2 & Fig.3)
Polythymidines (5, 10, 15, 20, 30 & 50 nucleotides)	1= dT 5; 2= dT 10; 3= dT 15; 4= dT 20; 5= dT 30; 6= dT 50
Oligonucleotides with 0 to 2 PS links	1= 0 PS; 2= 1 PS; 3= 2 PS
Oligonucleotides with 4 to 23 PS links	4= 4 PS; 5= 5 PS; 5= 5 PS; 6= 6 PS; 7= 7 PS; 8 = 8 PS; 9= 9 PS; 10= 10 PS; 11= 11 PS; 12= 12 PS; 13= 13 PS; 14= 14 PS; 15= 15 PS; 16= 16 PS; 17= 17 PS; 18= 18 PS; 19= 19 PS; 20= 20 PS; 21= 21 PS ; 22= 22 PS ; 23= 23 PS

CZE:

Acidic and basic BGEs were used to investigate the separation of the three samples. The results for acidic BGE were not convincing because most of the peaks were not well separated. As a result, basic BGE was selected for further investigation. As represented below, the separation was satisfying for polythymidines and oligonucleotides with 0 to 2 PS links in relatively short migration times. However, concerning the more complex mix of oligonucleotides with 4 to 23 PS links, more investigation have to be carried out in order to separate all the compounds.

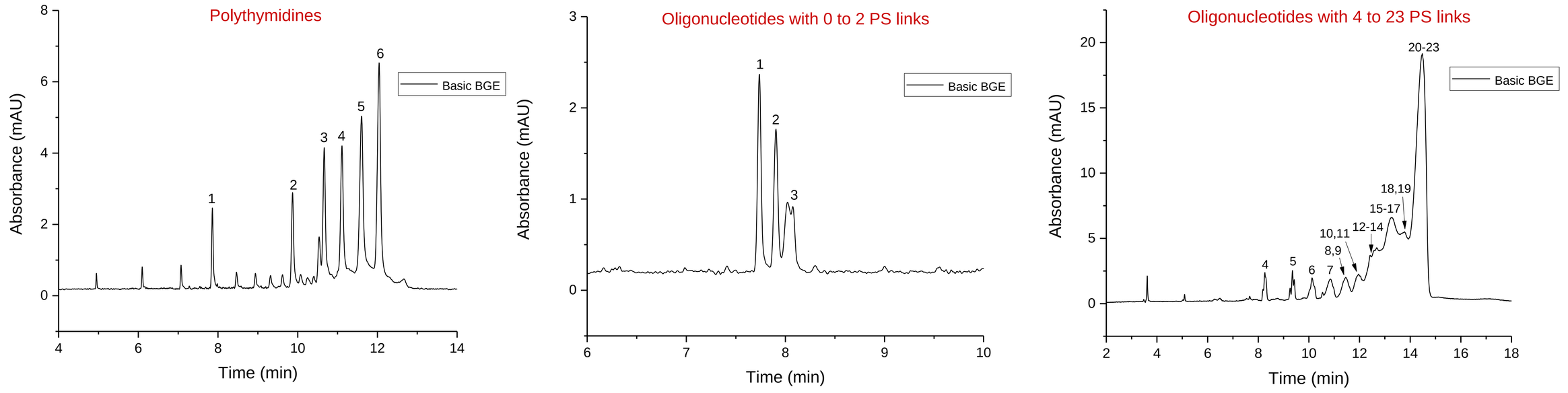

Fig.2 : Analysis of polythymidines, oligonucleotides with 0 to 2 PS links and oligonucleotides with 4 to 23 PS links by CZE-UV

HILIC mode:

The optimisation was mostly based on the mobile phase gradient without the addition of an ion pair reagent. The results showed a good separation of short-chain oligonucleotides with very satisfying resolutions (polythymidines and oligonucleotides with PS links). However, the separation was unsuccessful for longer compounds in both polythymidines and oligonucleotides with PS links.

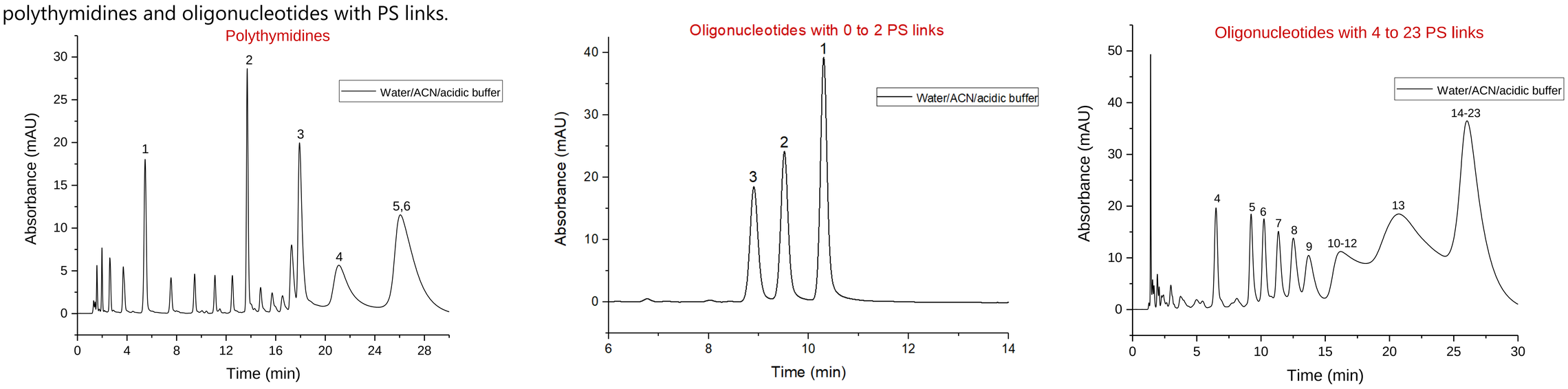

Fig.3 : Analysis of polythymidines, oligonucleotides with 0 to 2 PS links and oligonucleotides with 4 to 23 PS links by HILIC-UV

3. Conclusions

- **Polythymidines** → satisfying separation was obtained with basic BGE in CE in short analysis time. Concerning the HILIC mode, the short-chain oligonucleotides were nicely separated but the analysis of the long-chain oligonucleotides starting from 20 bases was challenging.
- **Oligonucleotides with 0 to 2 PS links** → baseline separation of the three analytes was obtained in HILIC mode.
- **Oligonucleotides with 4 to 23 PS links** → this sample is the most challenging to analyse due to its complexity brought by the amount of PS links. In HILIC mode, the short oligonucleotides were better separated than with basic BGE in CE. However, for the long oligonucleotides, with both techniques, no satisfying separation was obtained.

Based on the results so far, it is clear that both techniques are very promising for the analysis of oligonucleotides. Interestingly, both optimal conditions are compatible with mass spectrometry and do not contain ion-pair reagents, which opens new perspectives in terms of method sensitivity. In addition to that, we can conclude that CE seems to be complementary to HILIC mode for the analysis of long-chain oligonucleotides. More investigations have to be carried out to improve the separation of the compounds, especially long-chain oligonucleotides with PS links.

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