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EVALUATION OF THE GOVERNING PARAMETERS IN OLIGONUCLEOTIDES SEPARATION BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY USING FRACTIONAL FACTORIAL DESIGNS FOLLOWED BY SURFACE RESPONSE OPTIMIZATION

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The most common chromatographic methods for the analysis of oligonucleotides have been developed using ion-exchange liquid chromatography (IEXLC) and ion paring reverse phase liquid chromatography (IP-RPLC) with UV detection. Both techniques have demonstrated enough peak capacity to separate completely oligonucleotides up to 20 mer [1]. The raise in the usage of oligonucleotides in the therapeutic field in the last years, requires to couple a more sensitive and specific detector such as the mass spectrometer (MS) for the analysis of these molecules, their metabolites and impurities in complex matrixes. IP-RPLC using volatile buffers has been the only suitable method for coupling a MS detector; conversely, the high concentration of the ion paring reagent lowers the MS sensitivity. Hydrophilic interaction liquid chromatography (HILIC) has demonstrated to be a MS friendly chromatographic technique. HILIC methods are not popular for the analysis of oligonucleotides due to the poor peak capacities that have been obtained. Some methods have used polymer based zwitterionic stationary HILIC phases [2], obtaining with this greater peak capacities; however, this kind of chromatographic columns show les reproducibility between batches and are more expensive than silica columns, which are more suitable to implement in routine analysis.

A comprehensive study of HILIC using silica as stationary phase for the separation of oligonucleotides has been done. The separation of adenosine and thymidine oligonucleotides (up to 30 mer) by HILIC has been evaluated using fractional factorial designs. The separation has been performed in a 50 mm x 4.6 mm, Ascentis® HILIC column packed with silica of particle size 3.5 µm with an average pore diameter of 100 °*A*. A linear gradient elution using water and acetonitrile has been performed. The detection has been done at 260 nm using a single wave length detector. The studied separation parameters were: the column temperature; pH, ionic strength, initial water composition and gradient steepness of the mobile phase. The critical parameters where identified using screening designs, further a surface response has been build for the optimization of peak capacity.

The main governing factors in oligonucleotides (<20 mer) separation are the ionic strength of the mobile phase and the column temperature. However, the studied parameters do not have a high effect over oligonucleotides (>20mer). The effect of the column length, the particle size and the average pore diameter were also evaluated in order to achieve higher peak capacities for oligonucleotides (>20 mer). The HILIC shows a promising perspective for comprehensive (LC x LC) separation of complex oligonucleotides samples.

References

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