Oligonucleotides: The Next Big Challenge for Analytical Science

The Chromatographic Society Symposium on October 27th and 28th 2010 at GlaxoSmithKline, Amenities Centre, Ware, Herts.


Micellar electrokinetic chromatography was used to resolve diastereomers of oligonucleotides possessing several chiral phosphoramidite bridges. These materials were not resolved by conventional liquid chromatographic techniques.

Technological advancements employing novel nano switching and flow management have been applied to solve capillary switching in instrumentation to decode the human genome; arguably the biggest challenge ever to analytical chemistry! A multiplexed freeze-thaw switching principle and a distribution network were utilized to manage flow and sample transportation.


Publication Date (Web): September 3, 1998

The commercial development of oligonucleotide-based therapeutic drugs was almost abandoned for many years because some of the separation problems were considered insurmountable or not economically viable. However, in recent years interest has returned to this area as the great benefits of these drugs are being realised and licences granted.

Hence there is a growing need for the development of more sophisticated means for analysing them.

Although the present oligonucleotide synthesis is a reliable, fast and efficient process, the multi-step yield of target product is limited. The by-products of synthesis are mainly shorter oligonucleotides, termed failed sequences. For determining their products based on the size and configuration analysis of oligonucleotides using electrospray ionisation mass spectrometry (ESI MS) has been directly applied to real samples and successfully used.

On Friday 28th February 1992, Chris Bevan, Events Coordinator for the Chromatographic Society, explained the principles and applications of RNA chromatography. Dickman has elucidated the mechanism of the separation of RNA using ion pair reverse phase chromatography. The high-resolution separation of double stranded (ds) RNA was observed, in a similar manner to dsDNA under non-denaturing conditions. Moreover, the high-resolution separation of siRNA was observed at high temperatures (75 °C) in contrast to dsDNA. The presence of duplex region/secondary structures within the RNA remain at such temperatures, resulting in high resolution RNA separations.

The versatility of the application of RNA chromatography has been extended to a wide range of impurities, including the purification of synthetic oligonucleotides, RNA footprinting, the analysis of bacterial 16S RNA, the enrichment of small RNAs, including miRNAs and as an aid for the conformational analysis of RNA/RNA interactions. Furthermore, ion pair reverse phase chromatography has been interfaced to electrospray ionisation mass spectrometry (ESI MS) enabling the accurate molecular weight analysis of both synthetic and biological RNA.

Resident scientist Dr George O’Kane from Scienva, ESOL, Ware will give an overview of different radiolabelling strategies used for oligonucleotides based upon work from the scientific literature and experts in the field. This information will be used within ESOL to further develop synthetic strategies.

Also from big pharma, Adrian Akrar, Asta Zemica, will discuss pulmonary delivery of Oligonucleotides, focusing on analysis, formulation and regulatory considerations in early development. Kathy Ackley from Ginkgo will describe the use of orthogonal analytical methods for analysis of impurities in oligonucleotides.

And, from Roche Kulmbach GmbH, Bernhard Hall will present various analytical methods used in the siRNA drug development process, with a special focus on HPLC-MS. Examples of in-depth data analysis will be given and the challenges associated with process development, method development and transfer will be demonstrated using case studies. Setting of release specifications with regard to regulatory requirements will also be discussed.

George’s Nigel Richardson and Paul Newstead are looking at the practical implications for developing related impurity HPLC/UPLC methods for thiolated oligonucleotides. They will present the findings from their investigation.

Willem van Doren from Proxylab, Netherlands, will describe the development of UPLC methods for characterisation of synthetic oligonucleotides drugs. Synthetic oligonucleotides are short nucleic acid chains, typically 15-35 nucleotides long, which can direct gene-expression in a sequence specific manner. This class of therapeutic agents target the disease directly at the nucleus resulting in a broad therapeutic range. Although the present oligonucleotide synthesis is a reliable, fast and efficient process, the multi-step yield of target product is limited. The by-products of synthesis are mainly shorter oligonucleotides, termed failed sequences. For determining activity and safety of the oligonucleotide agents, analytical methods are necessary to identify and quantify the failed sequences and other impurities to as low level. For development of these methods state-of-the-art separation technology such as ultra performance liquid chromatography (UPLC) coupled to UVP and mass spectrometry has been applied. In his presentation, the process of analytical method development and qualification for oligonucleotide products will be outlined.

Although oligonucleotides lay in a niche analytical area, the successful analytical solutions will often become applicable in other difficult areas.

Any analyst who wishes to stay at the forefront of the subject will benefit from the wisdom shared at this symposium and should make every effort to attend.