

# Chromatography Focus

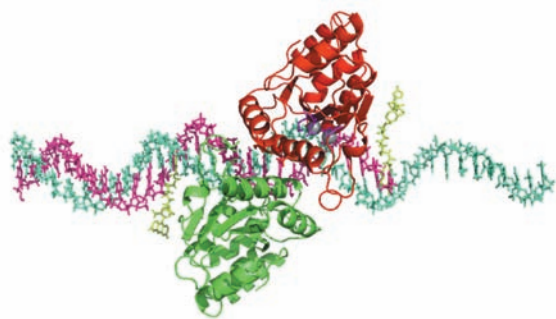
## Oligonucleotides: The Next Big Challenge for Analytical Science

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



***It is now over forty years since I started my career in analytical chemistry and during that time I have been privileged to witness many significant advances in the science and technology that underpin this important branch of chemistry. Most of these advances have arisen as a result of challenges from trace level analyte determinations, complex matrices, subtle structural idiosyncrasies and the omnipotent demand to make everything run faster and more reliably.***

***One of the most challenging analyses I have encountered personally was almost 20 years ago whilst developing chromatographic and electrophoretic methods for the separation and quantitative measurement of unnatural synthetic oligonucleotides, as part of developing antisense drugs.***



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*Resolution of diastereomeric phosphoramidate bridged unnatural oligonucleotides by micellar electrokinetic chromatography.*

*Journal of Chromatography A*

*Volume 636, Issue 1, 23 April 1993, Pages 113-123*

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

Technological advancements employing novel nano switching and flow management means 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.

*Journal of Chromatography A*

*Volume 697, Issues 1-2, 21 April 1995, Pages 541-548*

*20th International Symposium on Chromatography*

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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 great 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 ever more sophisticated means for analysing them.

This highly topical symposium has attracted current international specialists and pharma insider experts in this area who will be describing their work and revealing the analytical challenges they face.

Notable amongst these, we have:

Vesela Encheva from LGC, Middlesex, describing the characterisation of oligonucleotide mixtures using electrospray ionisation mass spectrometry (ESI MS)

Her study demonstrated the utility of ESI MS for the detection and base composition determination of oligonucleotides ranging in size from 80 to 200 base pairs. Her methodology has been directly applied to real samples and successfully used for genotyping human mitochondrial DNA.

From Dionex UK, Ken Cook will describe high resolution ion exchange separations of a broad spectrum of oligonucleotides with automated off-line desalting for MS.

Among the primary desires of oligonucleotide workers are separation of their products based on the size and modifications to the oligos, be they on the base, the ribose or the backbone. All are employed, and all have been demonstrated with high resolution anion exchange separations. Particular reference will be made to aberrant linkage isomers of RNA, thioation and an automated off-line desalting protocol for MS which can be introduced as a second dimension separation. Some of the common impurities found in oligonucleotides, such as linkage isomers can be isobaric and so will not be addressable by standard LC-MS. Separation of a broad spectrum of oligonucleotides is possible and examples of control of the separation by pH, temperature and counter ion used is shown. Introduction of polymeric monoliths for oligonucleotide separations is also demonstrated.

Mark J Dickman from ChELSI Institute,, University of Sheffield, Sheffield UK, will explain 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 ssRNA was observed at high temperatures (75 °C) in contrast to ssDNA. The presence of duplex regions/secondary structures within the RNA remain at such temperatures, resulting in high resolution RNA separations. The retention time of the nucleic acids reflects the relative hydrophobicity, through contributions of the nucleic sequence and the degree of secondary structure present. The versatility of the application of RNA chromatography has been extended to a wide range of important applications including; the purification of synthetic oligoribonucleotides, RNA foot printing, the analysis of bacterial 16S rRNA, 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 Okafo from Scinovo, GSK, 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 GSK to further develop synthetic strategies.

Also from big pharma, Nadim Akhtar, Astra Zeneca, will discuss pulmonary delivery of Oligonucleotides; focussing on analytical, formulation and regulatory considerations in early development.

Kathy Ackley from Girindus will describe the use of orthogonal analytical methods for analysis of impurities in oligonucleotides.

And, from Roche Kulmbach GmbH, Bernhard Noll 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.

GSK's Nigel Richardson and Paul Newstead are looking at the practical implications for developing related impurity HPLC/UHPLC methods for thiolated oligonucleotides. They will present the findings from their investigations.

William van Dongen from Proxylab, Netherlands, will describe the development of UPLC methods for characterisation of synthetic oligonucleotide 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 diseases literally 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 at very low level. For development of these methods state-of-the-art separation technology such as ultra performance liquid chromatography (UPLC) coupled to UV 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.