Oligonucleotide Separations Using Ion-Pairing Reversed Phase Liquid Chromatography

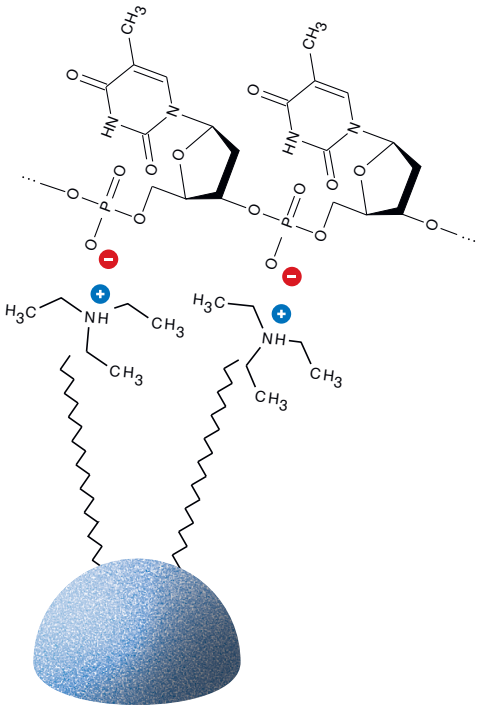
Introduction

Traditionally, reversed phase liquid chromatography was incapable of retaining highly polar compounds due to the non-polar nature of the stationary phase modifications used. Ion pairing reversed phase chromatography (IP-RP) is an alternative approach which uses ion pairing reagents to act as a mediator between the polar target molecules and the non-polar stationary phase. Such ion pairing reagents are typically alkylsulfonates, alkylammonium salts or similar compounds such as triethylamine (TEA).

While the exact mechanisms of the interactions are still under investigation, there are currently two proposed models that describe the interactions which occur and these may well be co-existing in reality: The partition model assumes that the ion pairing reagent and the target compound interact first, forming an ion pair that is less polar in nature in comparison to the target substance by itself, and in turn undergoes retention interactions with the stationary phase.

The adsorption model on the other hand is based on an interaction between the ion pairing reagent and the stationary phase occurring first. In this case, the hydrophobic alkyl chain of the ion pairing reagent would be attached to the alkyl chains of the stationary phase modification, presenting its potentially ionic site to the target compounds in the mobile phase. This mechanism may be interpreted as a pseudo ion exchange surface, which combines the advantages of ion exchange resins (separation characteristics) with those of RP-HPLC phases (greater efficiency, pressure stability).

The use of ion pairing chromatography is the new gold standard for the separation of peptides, oligonucleotides and other molecules with charged functional groups. Facing different objectives, the IP-RP conditions can be optimised for analytical separations or preparative scale purifications.



Separation of oligonucleotides

The backbone of oligonucleotides has a high density of negative charges. This makes the use of RP chromatography for the isolation of oligonucleotides complicated. The principles of ion pairing reversed phase chromatography are the basis for the separation of oligonucleotides based on the interaction of its hydrophobicity.

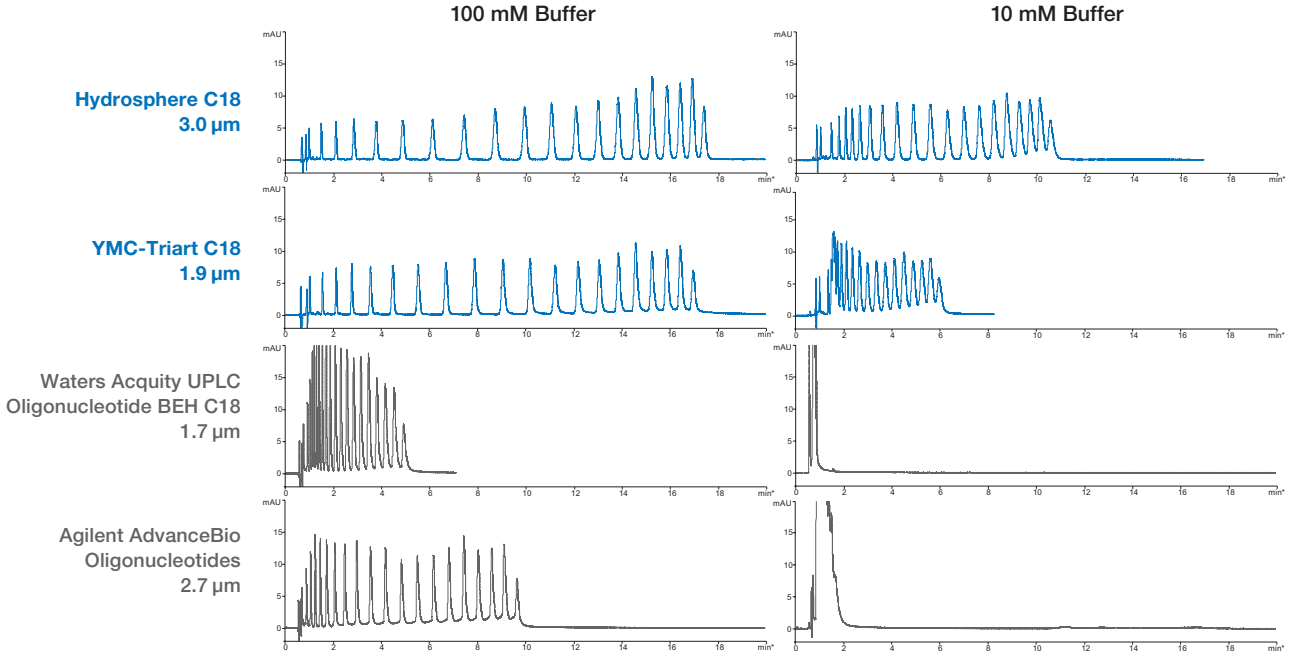
There are numerous buffer types and possible combinations available which have a significant effect on the actual separation characteristics. Also, the use of IP-RP compatible stationary phases is crucial for the development of efficient separation processes.

The most appropriate way to optimise the conditions for the mobile phase and the stationary phase is a comprehensive screening. Every separation process has its own requirements and also every oligonucleotide is different.

Stationary phase screening

The selectivity of a stationary phase is the strongest tool in liquid chromatography. The choice of the most suitable stationary phase is even more important for IP-RP separations. The bonding chemistry is critical as to whether the retention for the highly polar compounds can be increased efficiently when applying IP-RP conditions. The required concentration of the buffer also depends on the bonding chemistry of the stationary phase used.

The comparison in Fig. 1 illustrates the effect of the stationary phase used for the retention ability for polar compounds. The YMC phases Hydrosphere C18 and YMC-Triart C18 allow much higher retention for the oligonucleotide sample compared to two other phases. This behaviour is shown for two different buffer concentrations. Therefore, the use of IP-RP compatible stationary phases is a must, and a comprehensive screening during the development of new oligonucleotide separations is highly recommended.



Column:	50 x 2.1 mm ID	Detection:	UV at 260 nm
Eluent:	A) 100 mM or 10 mM triethylamine-acetic acid (pH 6.0)	Temperature:	35 °C
	B) Eluent A/acetonitrile (80/20)	Sample:	Oligodeoxythymidylic acid [$d(pT)_{2-20}$]
Gradient:	50–65%B (0–20 min)	Injection :	2 µl (5 nmol/ml)
Flow rate:	0.21 ml/min		

Fig. 1: Comparison of d(pT)2-20 separation using different C18 phases.

Mobile phase screening

The effective selectivity is a combination of the stationary phase used and the mobile phase. For the separation of oligonucleotides, a suitable buffer is required to act as the ion pairing reagent. There are different buffer systems available. The buffers used need to be available in large scale quantities and mustn't be too expensive considering the intended use in the preparative chromatography. This is why the gold standard for the analytical separation of oligonucleotides, HFIP, is not a feasible option for the use in large scale processes for the isolation of oligonucleotides.

In Fig. 2, two different typical buffers are tested for the separation of oligonucleotides. In case of di-n-butylammonium acetate (DBAA), two different concentrations were additionally tested. It demonstrates the effect of the buffer used as well as the concentration on the retention ability and the peak shape. Generally, a lower concentration is preferred with regard to the subsequent steps in the purification process.

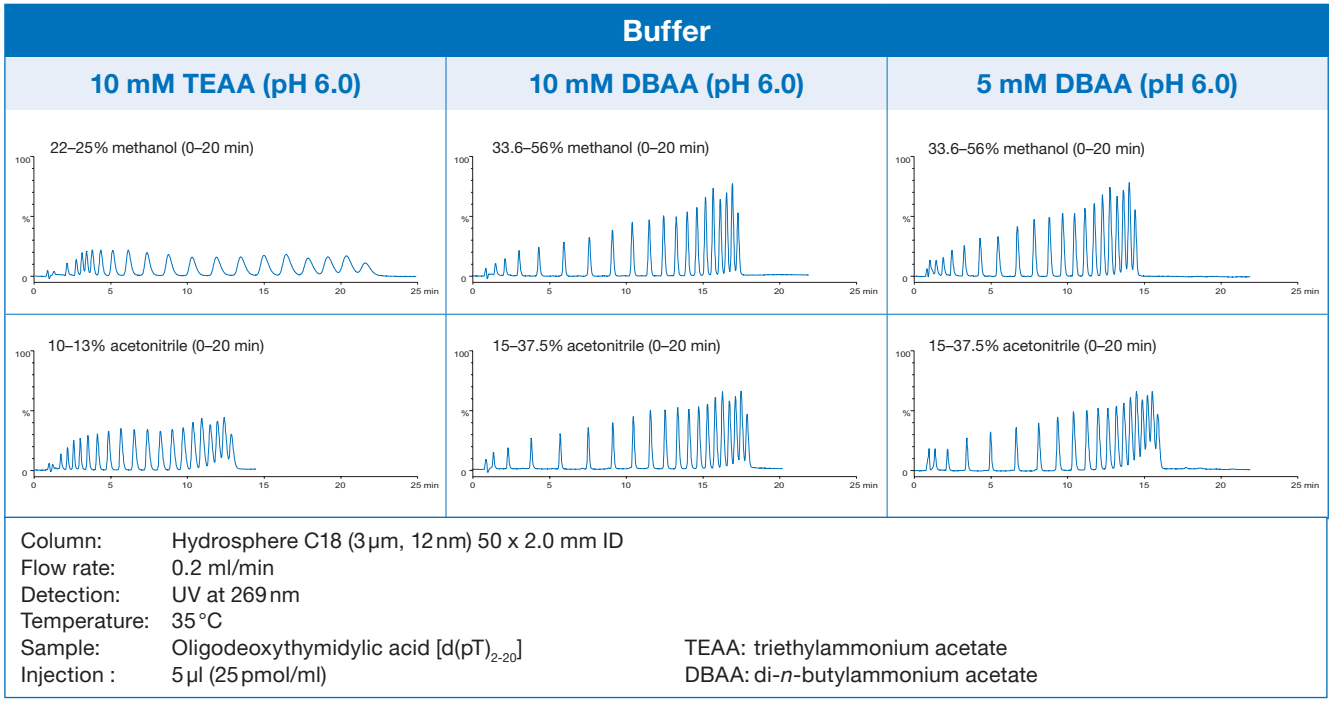


Fig. 2: Comparison of separation using different ion-pairing buffers and organic solvents.

How to choose a suitable RP phase?

YMC-Triart Prep materials and the YMC*Gel ODS-AQ-HG materials are the ideal choice for IP-RP methods. The selectivity with its hydrogen bonding capacity is perfectly suited for the purification of peptides, oligonucleotides and other biomolecules. The method described using IP-RP conditions for the purification of peptides, oligonucleotides and other charged molecules has been applied on an industrial scale with YMC materials in the pharmaceutical industry.

Due to the hybrid-silica base particles of the YMC-Triart Prep materials, the usable pH range is extended compared to silica-based materials from typically 2.0–7.5 to 2.0–10.0. The ability to apply alkaline conditions within the mobile phase allows the development of purification processes at high pH values and it allows the use of alkaline cleaning procedures for an increased lifetime of the packed column. This improves the cost-efficiency of the production for new API's.

Specifications

	YMC-Triart Prep C18-S	YMC-Triart Prep C8-S	YMC-Triart Prep Bio200 C8	YMC-Triart Prep Phenyl-S	YMC*Gel ODS-AQ-HG
Base material	inorganic/organic hybrid silica				silica
Modification	C18	C8	C8	Phenyl	C18
Particle size [µm]	7, 10, 15, 20	10, 15, 20	10	10	10, 15, 20, 50
Pore size [nm]	12	12	20	12	8, 12, 20
Specific surface area [m^2/g]	360	360	proprietary	360	330
pH range	2.0–10.0	2.0–10.0	2.0–10.0	2.0–10.0	2.0–7.5

Ordering Information

YMC-Triart Prep C18-S			YMC-Triart Prep C8-S		
Pore size [nm]	Particle size [µm]	Product Code	Pore size [nm]	Particle size [µm]	Product Code
12	7	TAS12S07	12	10	TOS12S11
	10	TAS12S11		15	TOS12S16
	15	TAS12S16		20	TOS12S21
	20	TAS12S21			

YMC-Triart Prep Bio200 C8 NEW			YMC-Triart Prep Phenyl-S NEW		
Pore size [nm]	Particle size [µm]	Product Code	Pore size [nm]	Particle size [µm]	Product Code
20	10	TOB20S11	12	10	TPS12S11

YMC*Gel ODS-AQ-HG		
Pore size [nm]	Particle size [µm]	Product Code
12	10	AQG12S11
	15	AQG12S16
	20	AQG12S21
	50	AQG12S50

Other particle and pore size combinations are available on request.

More information about the benefits of the YMC-Triart Prep materials can be found on the YMC website www.ymc.eu or please contact your YMC representative.

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 2064 427-0 · Fax +49 2064 427-222 · Email info@ymc.eu · www.ymc.eu