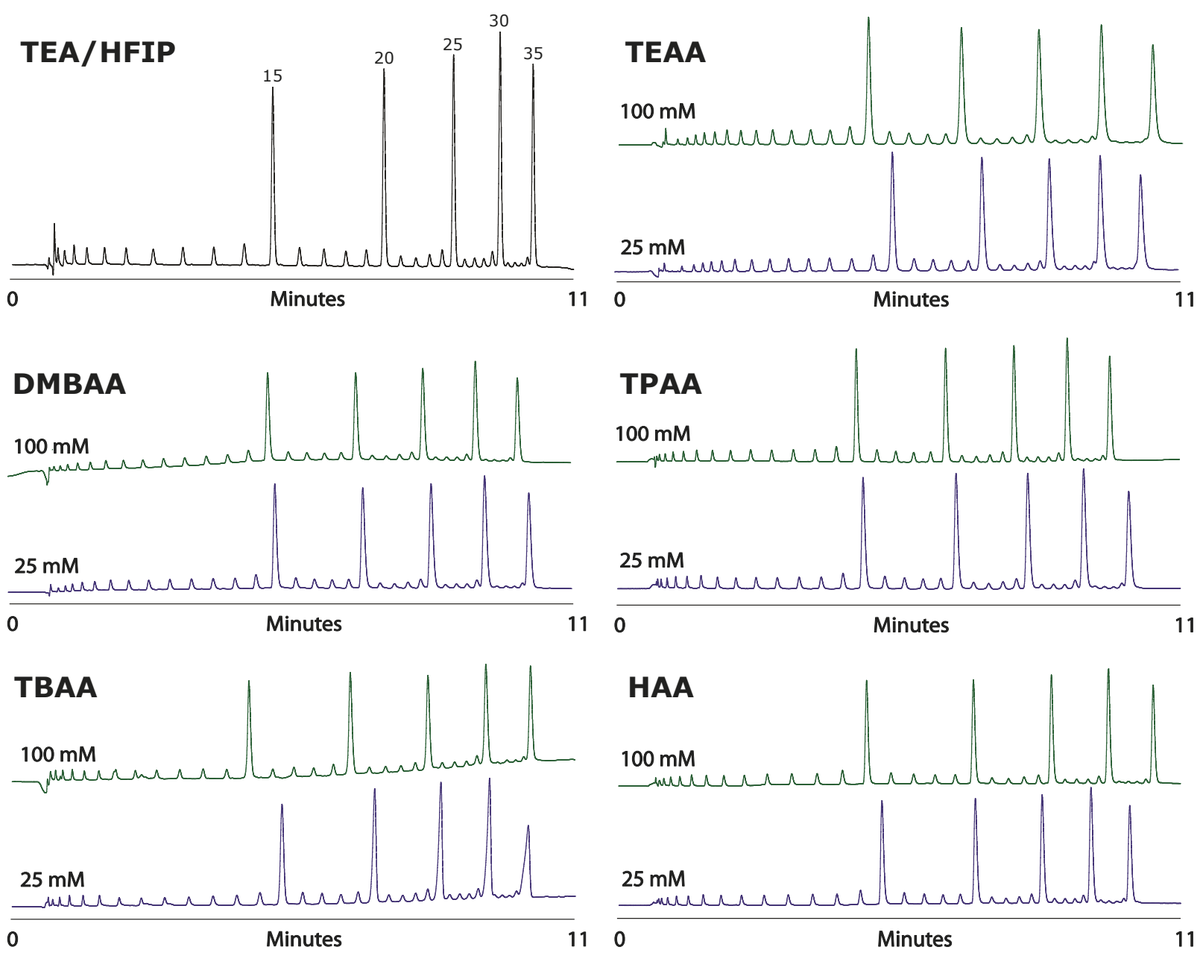
ION-PAIRING SYSTEMS FOR REVERSED-PHASE CHROMATOGRAPHY OF OLIGONUCLEOTIDES

Sean M. McCarthy, Ananya Dubey, Martin Gilar
Waters Corporation, 34 Maple Street, Milford, MA 01757

INTRODUCTION

Synthetic oligonucleotides are produced using highly efficient solid phase processes which have been optimized to produce very high yields, often above 99% per synthetic step. Following synthesis, it is common to assess purity of the final product. Commonly, this is accomplished using chromatographic methods, such as ion exchange (IEX) or ion pairing reverse phase chromatography (IP-RP). Recently, IP-RP methods have become more popular due to their compatibility with MS detection and equvilent or better resolution to IEX in a fraction of the time. In this presentation we will discuss a systematic comparison if the resolving power and MS compatability of ion-pairing systems for oligonucleotide analysis. Our study focuses on the comparison of a variety of alkyl amines of varying hydrophobicity. To evaluate each amines suitability for IP-RP separations, we compared separations at different concentrations by determining their peak capacities for a homopolymer oligonucleotide separation. We selected the higher performing amines and performed separations of heteropolymers to access each systems ability to separation by charge while minimizing hydrophobic contribution. Our results show that both triethylamine/hexafluoroisopropanol (TEA/HFIP) and hexylammonium acetate (HAA) provide the greatest peak capacities and separations largely driven by charge. We found that HFIP is best suited for MS analysis.

HOMOMERIC OLIGONUCLEOTIDE SEPARATIONS



- **Separations were accomplished with a Waters ACQUITY UPLC® System using a Waters Oligonucleotide Separations Technology column (ACQUITY UPLC® OST C18, 1.7µm, 2.1x50) maintained at 60 °C. On column loading was 20 pmol/oligo of Waters MassPREP™ OST standard. Elution conditions were adjusted so 15-mer eluted at ≈ 5 minutes and 35-mer eluted at ≈ 10 min.**

- **More hydrophobic IP agents required higher ACN content for oligo elution.**
- **Mobile phases were prepared by the addition of equimolar ratios of acetic acid and appropriate base. The pH was adjusted to ≈ 7.0 by the addition of either acid or amine as needed.**

- **Most mobile phase modifiers tested provide acceptable resolution at high concentrations, ca. 100 mM.**
- **More hydrophobic IP agents such as HAA, DMBAA, and TPAA perform best and maintain their resolving ability across the range of 15-35 mers at lower modifier concentrations.**
- **All modifiers yield similar MS response with the exception of TEA/HFIP which yields the best response**

CONCLUSIONS

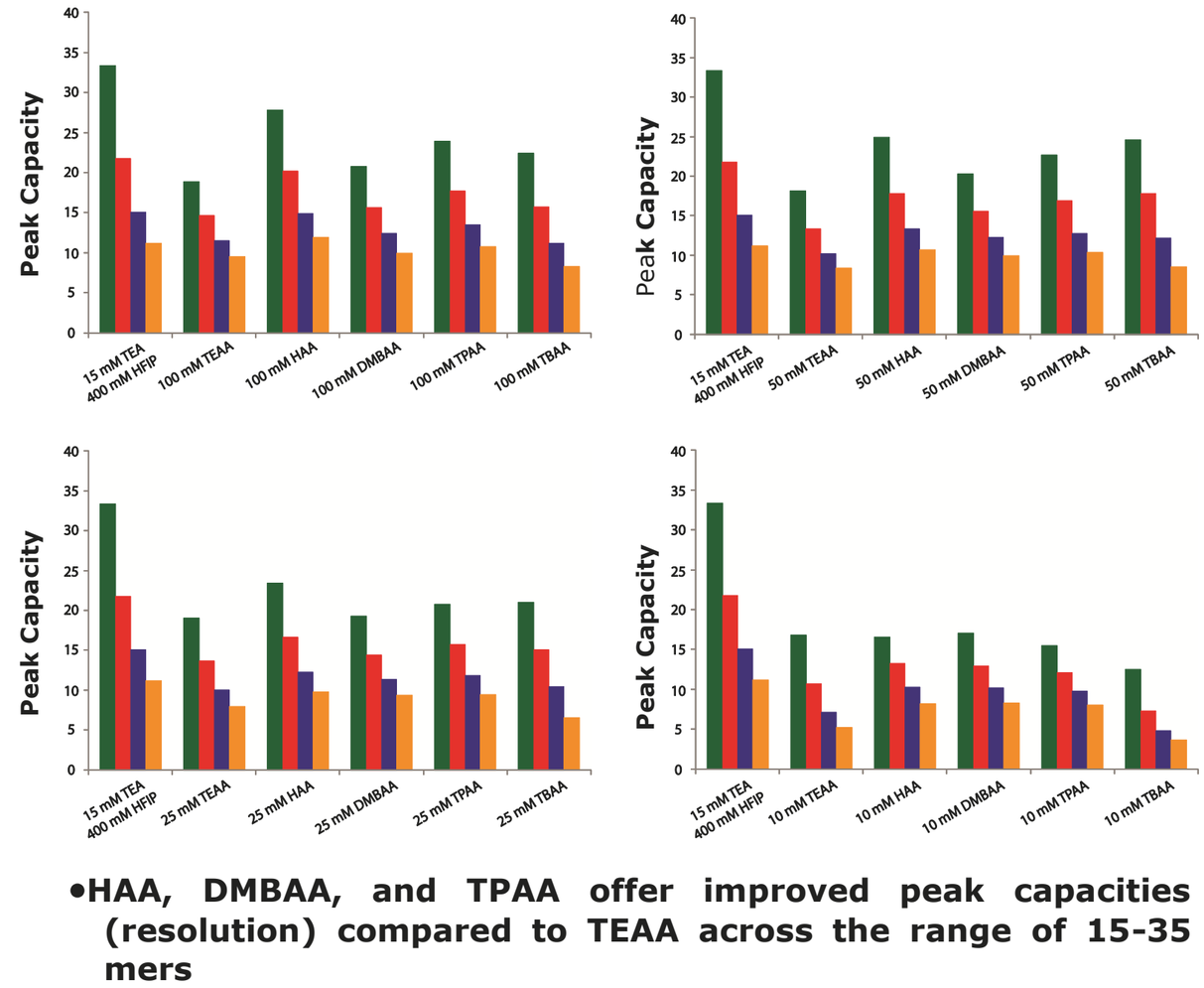
- **HAA, TPAA, and DMBAA provide good resolution of oligo dT,**
- **HAA and TPAA gives predictable retention order of heteromeric oligonucleotides, but HAA provides better resolution.**
- **Increased ion-paring efficiency makes the retention pattern of heteromeric oligos more regular.**
- **Higher ACN content minimizes RP effects on oligonucleotide retention further contributing to regularity in retention.**

COMPARISON OF RESOLUTION FOR ION-PAIRING SYSTEMS

- **Peak capacity reflects how many peaks could fit between two selected mers.**
- **Peak capacity (*P*) was calculated from equation below.**

$$P = 1 + \frac{t_2 - t_1}{(w_2 + w_1)/2}$$

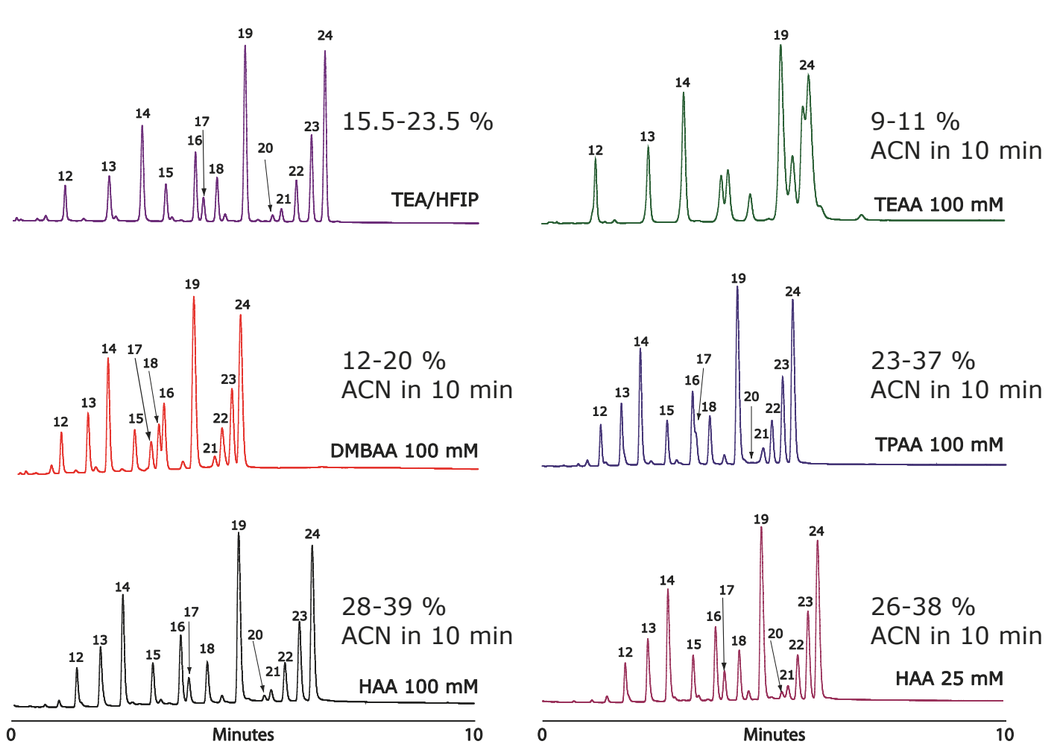
t_1 and t_2 are retention times of two selected peaks, w_1 and w_2 are peak widths of peaks measured at 32.4% peak height (3σ).



- **HAA, DMBAA, and TPAA offer improved peak capacities (resolution) compared to TEAA across the range of 15-35 mers**
- **Peak capacity declines less rapidly for acetate buffers compared to TEA/HFIP**
- **All acetate buffered modifiers yield similar MS response**

HETEROMERIC OLIGONUCLEOTIDES

- **IP RP LC can yields irregular retention of heteromeric oligonucleotides. More efficient IP agents will provide stronger charge-to-charge interaction with oligos providing more regular retention.**
- **We prepared a sample of synthetic 24-mer, 19-mer and 14-mers (5'- CCC CTT GGT TAA CCA AGG TTC CAA-3') and digested the mixture with phosphodiesterase II to test regularity of our IP agents.**



TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS