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Analysis of oligonucleotides by ion-pair hydrophilic interaction liquid chromatography/electrospray ionization mass spectrometry

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Synthetic oligonucleotides are widely used in the polymerase chain reaction (PCR) as DNA primers or in molecular biology as probes to screen for diseases, viral infections, and to identify genes. Sensitive and selective methods have always been demanded for the characterization of oligonucleotides, especially, when the oligonucleotides are applied as therapeutics. Ion-pair reversed-phase (IP-RP) liquid chromatography has been commonly used for the analysis of oligonucleotides, but ion suppression is a major problem when coupling with electrospray ionization mass spectrometry (ESI-MS). Although the introduction of hexafluoroisopropanol (HFIP) in the mobile phase has improved MS detection sensitivity of oligonucleotides, this mobile phase system results in a severe problem with adducts formation particularly if large oligonucleotides are analysed. An alternative chromatographic approach, hydrophilic interaction liquid chromatography (HILIC), was recently employed for the analysis of oligonucleotides. It provided enhanced MS sensitivity with fewer adducts but lacked chromatographic resolution for some oligonucleotides. Here, we improve chromatographic resolution while maintaining MS sensitivity by adding ion-pairing reagent, triethylammonium acetate (TEAA), into the HILIC mobile phase. The IP-HILIC approach produces lower retention capacity and has the added benefit of providing simpler MS spectra, with fewer charge states, when comparing with HILIC. We suggest a mechanism for ion-molecule interactions in IP-HILIC.

Biography

Lingzhi Gong's general research interests include "Mass spectrometry based analysis of small molecules and bio-molecules (nucleic acids, peptides, proteins) through hyphenated to a chromatographic method (mainly liquid chromatography), and research into chromatographic retention mechanisms". His current work focuses on "Characterizing single- and double-stranded DNA/RNA, and protein/peptide-DNA crosslinking complex using hyphenated liquid chromatography and electrospray mass spectrometry (LC-ESIMS)

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