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Note d'application

Evaluating IonHance Hexafluoroisopropanol (HFIP) for Enhanced LC-MS Oligonucleotide Analysis

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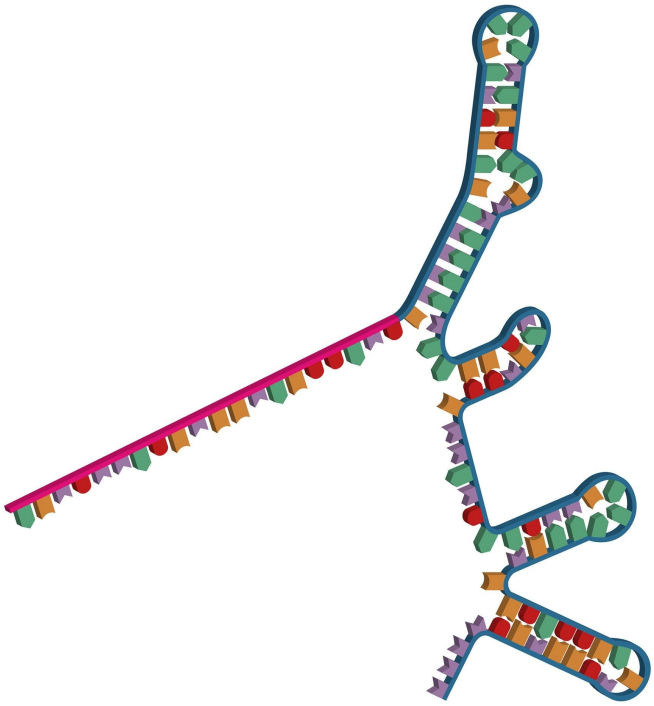
Ce document est une note d'application et ne contient pas de section détaillée concernant l'expérimentation.

Abstract

To demonstrate the use of IonHance Hexafluoroisopropanol (HFIP) as a mobile phase additive to improve ionization and overall spectral quality for oligonucleotide LC-MS analysis.


IonHance Hexafluoroisopropanol
Part No. 186010781-1
Exp. Date 17-Apr-2027
Tel. +1-508-482-2000
Full labeling information for this product is provided on the outside package

IonHance™



Introduction

LC-MS analysis is critical for the precise separation, identification, and quantitation of RNA species such as siRNA, antisense oligonucleotides (ASOs), CRISPR single guide RNA (sgRNA), and messenger RNA (mRNA). Ion-pair reversed-phase (IP-RP) is the most common method for LC-MS of oligonucleotides and it often requires the use of volatile buffers made from a combination of alkyl amines and HFIP, which enhance separation efficiency while ensuring compatibility with mass spectrometry. It is important to consider the quality of mobile phase additives and their impact on data interpretation, as challenges can arise even with LC-MS grade reagents. Oligonucleotide samples can readily bind to trace metal impurities originating from mobile phase reagents and additives, complicating data interpretation attributed to increased adduct formation. IonHance HFIP was developed to address these challenges by paying critical attention to manufacturing parameters and striving for a more stringent level of purity certification.

HFIP can enhance MS signal strength by up to ten orders of magnitude when compared to traditional acid buffers.[1] It is a highly volatile acid that readily evaporates during ionization. We have spent time closely examining this increasingly critical reagent.

In addition to HFIP quality, ensuring a consistent supply of HFIP is crucial for maintaining reliable workflows and reproducible results. Any disruption in a critical reagent's availability can negatively impact sample throughput and project turnaround times. IonHance HFIP addresses both performance and supply concerns by offering the highest purity reagent with reliable production and availability from a trusted manufacturer, ensuring that laboratories can consistently meet analytical demands without risk of supply interruptions.

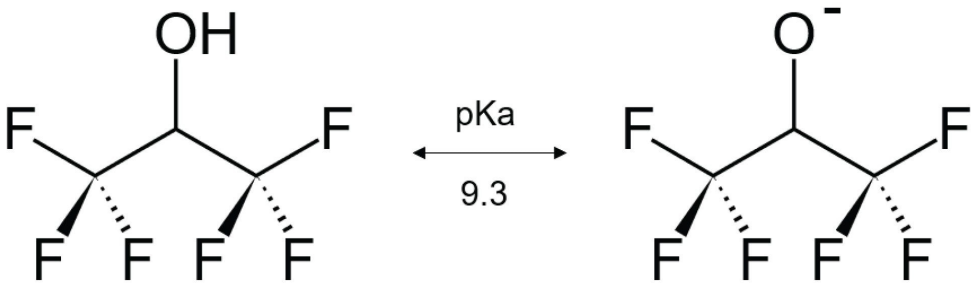


Figure 1. Chemical Structure of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and the pKa.[1]

Experimental

The Solution

IonHance HFIP (p/n: 186010781) is a refined, highly purified form of hexafluoroisopropanol (HFIP) that provides exceptional performance for LC-MS analysis. The proprietary distillation process supports a tight metals specification (<100 ppb Na and <100 ppb K) for an LC-MS grade HFIP reagent. This ensures that metal impurities are minimized such that improvements in MS signal clarity and accuracy can be achieved.

It is recommended to use 50 to 100 mM HFIP in the mobile phase. These reagent concentrations provide a balance between optimal chromatographic resolution and high MS signal sensitivity. The Ionhance HFIP vial contains an overfilled 10 mL volume of reagent for routine preparation of 1L mobile phases. This concentration (0.5–1% v/v HFIP) range allows for the separation of long-chain oligonucleotides (up to 100-mers) including complex backbone, sugar, and base modifications. Care should be given to minimize the exposure of mobile phase to oxygen, and fresh mobile phases should be prepared regularly.[2]

Results and Discussion

High Quality HFIP

An ICP-MS analysis was conducted to assess the metal content of IonHance HFIP following its manufacturing and purification processes. Table 1 lists the sodium (Na) and potassium (K) concentrations for five replicates from two production batches of IonHance HFIP post-purification. The results indicate that sodium and potassium levels are well below the <100 ppb specification outlined in the IonHance HFIP Certificate of Analysis.

To further illustrate the impact of the IonHance HFIP manufacturing process on obtaining quality MS results, we have performed an analysis on a 10 to 60-mer single-stranded DNA (ssDNA) ladder (p/n: 186009449 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009449-ssdna-10-to-60-ladder.html>) using an ACQUITY™ Premier Oligonucleotide BEH™ C_{18} 300 Å 1.7 µm Column (p/n: 186010539 < https://www.waters.com/nextgen/global/shop/columns/186010539-acquity-premier-oligonucleotide-beh-c18-column-300-a-17--m-21-x-.html>). Equivalent mobile phases comprised of N,N-diisopropylethylamine (DIPEA) and 1% (v/v) HFIP (pre and post-purification) were prepared. The mass spectrum of the 10-mer oligonucleotide

was then compared. It was observed that as purity increased more signal was observed in the form of the deprotonated base peak ion. Furthermore, sodium, and potassium adduct formation was found to be reduced up to two times at the $[M-2H^+]^{2-}$ charge state. Overlaying and normalizing the spectra to the base peak height demonstrated the relative reduction in sodium and potassium adduct formation when using purified IonHance HFIP.

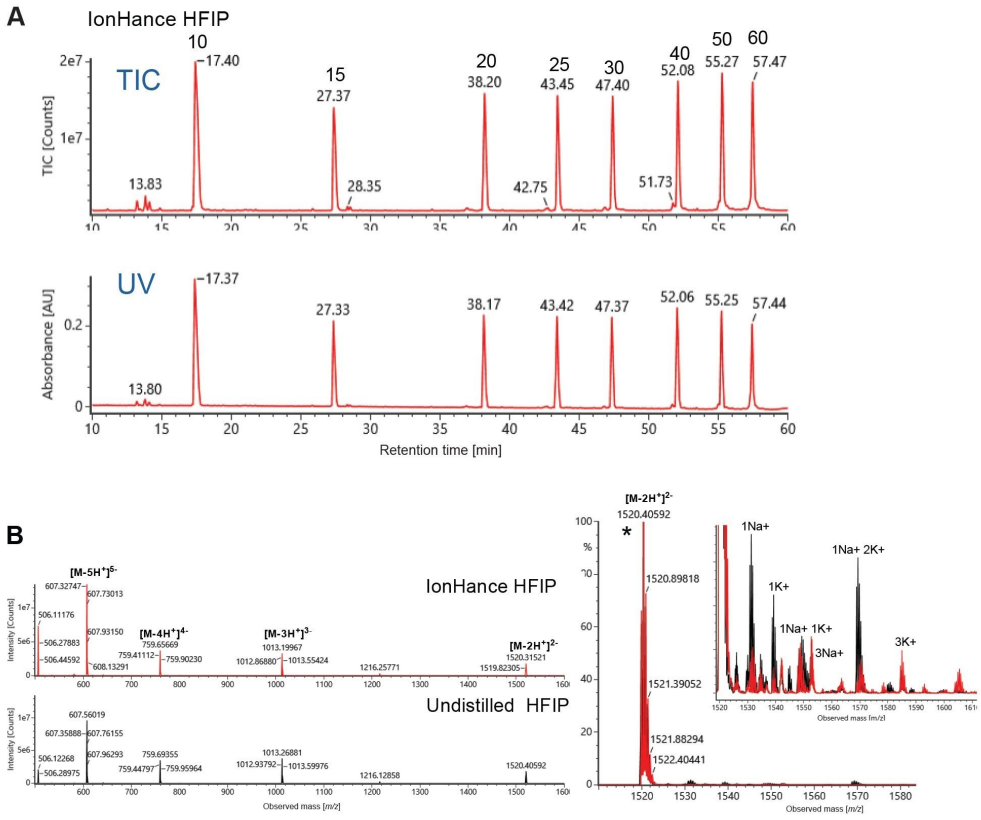


*Figure 2.IP-RP-LC-UV-MS analysis of 10–60 mer ssDNA ladder (p/n: 186009449). A. TIC (Total ion chromatogram) and UV chromatograms of ssDNA ladder following LC-MS are shown. B. Mass spectra of 10-mer eluting at 14.4 minutes comparing the charge state distribution of IonHance HFIP (red) to undistilled HFIP (black). The right-side plot is normalized to the [M-2H+]2- base peak (denoted by *) highlighting the reduced sodium and potassium adducts (red vs black trace of mass spectra) for distilled version compared to undistilled version of the IonHance HFIP.*

These benefits are also observed when comparing IonHance HFIP to alternative commercially available LC-MS

grade HFIP reagents. For comparison, an LC-MS system was flushed overnight with a 25:25:25:25 mixture of MeOH:ACN:IPA:H2O formic acid acidified. Samples were analyzed with IP-RP mobile phases containing Vendor S HFIP. Before switching over to IonHance HFIP, the LC-MS system was again flushed overnight with the aforementioned mixture. The same mobile phase bottle with the Waters™ Certified glass was used after rinsing twice with double distilled H2O. Certified LDPE container (p/n: 186009110 < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186009110-certified-ldpe-container-1000-ml.html>) may also be considered for mobile phase storage. Figure 3 investigates a 100 mer oligonucleotide analyte from the corresponding LC-MS runs. A raw mass spectrum, zoomed viewed of the base peak charge station ion, and a deconvoluted mass spectrum are shown as obtained with each example reagent. Significantly improved data quality was achieved with use of the IonHance HFIP.

IonHance HFIP	Sodium (ppbw)	Potassium (ppbw‡)
Batch P17-1	6.3	4.0
Batch P17-2	<5	1.2
Batch P17-3	<5	1.1
Batch P17-4	<5	2.0
Batch P17-5	<5	2.1
Batch P13-1	6.4	2.9
Batch P13-2	5.3	1.9
Batch P13-3	6.4	3.1
Batch P13-4	6.9	4.0
Batch P13-5	4.9	3.8
Average	**5.6**	**2.6**

‡parts per billion by weight (ppbw)

Note: (<) indicates below LOD

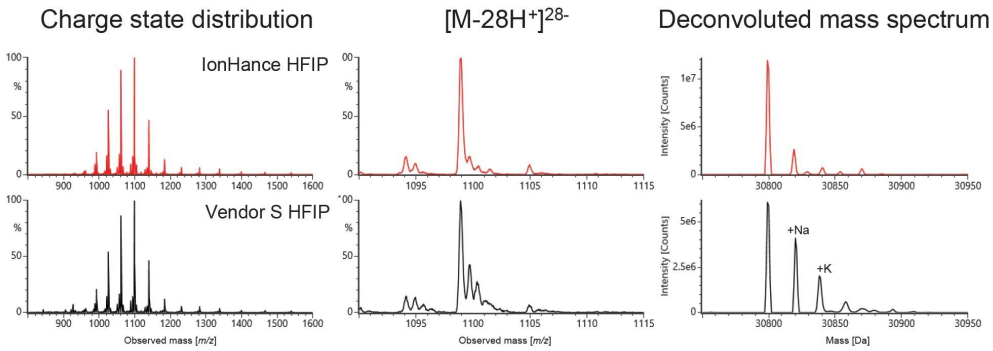


Figure 3. IP-RP-LC-MS analysis of 100 mer oligonucleotide standard on an ACQUITY Premier Oligonucleotide BEH C18 300 Å 1.7 µm 2.1 x 50 mm Column (p/n: 186010539). The deconvoluted mass spectrum highlighting observed adducts for HFIP Vendor S and IonHance HFIP is shown.

Conclusion

LC-MS data quality can be directly correlated to the purity of mobile phase reagents. IonHance HFIP (186010781) is a highly purified mobile phase additive designed to enhance the performance of oligonucleotide analysis via LC-MS. The proprietary purification process ensures that IonHance HFIP maintains exceptionally low sodium (<100 ppb) and potassium levels (<100 ppb). In turn, a mobile phase prepared with IonHance HFIP reliably yield mass spectra with low levels of sodium and potassium adducts. This reduction leads to improved mass spectra data quality, reduced adduct formation, and enhanced signal clarity.

In comparative studies using a 10 and 100 mer oligonucleotide standard, IonHance HFIP has been found to provide notable reductions in sodium and potassium adducts. This improvement in data quality simplifies interpretation and increases confidence, making IonHance HFIP an excellent choice for laboratories regularly performing ion pair reversed phase LC-MS analyses.

References

1. Donegan, M., Nguyen, J. M., & Gilar, M. (2021). Effect of Ion-Pairing Reagent Hydrophobicity On Liquid Chromatography and Mass Spectrometry Analysis of Oligonucleotides. *Journal of Chromatography* A, 1666, 46286.

2. Guilherme J. Guimaraes, Jack G. Saad, Vidya Annavarapu, and Michael G. Bartlett (2023) Mobile Phase Aging and its Impact on Electrospray Ionoization of Oligonucleotides.

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IonHance MS-Grade Mobile Phase Additives and Buffers < https://www.waters.com/nextgen/global/products/standards-and-reagents/ionhance-ms-grade-mobile-phase-additives-and-buffers.html>

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