Accelerated Stability Assessment Program (ASAP) for the Rapid Determination of Shelf Life for Oligonucleotides

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Introduction

- Oligonucleotide therapeutics including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and aptamers are generally manufactured as lyophiles or aqueous solutions in vials or pre-filled syringes.
- Predominant degradation pathways include hydrolysis and oxidation.
  - Sensitivity to temperature, humidity, and oxygen has been demonstrated.
- Accelerated Stability Assessment Program (ASAP) case study performed using a lipid-conjugated ASO reference standard provided by Waters Corporation.

  5' d 5-Pal-*-MOE-MeC-*-MOE-G-*-MOE-MeC-*C*G*A*T*A*A*G*G*T*A*-MOE-MeC-*-MOE-A-*-MOE-MeC 3'

  * = phosphorothioate bond (sulfur replacing oxygen in phosphate linkage)

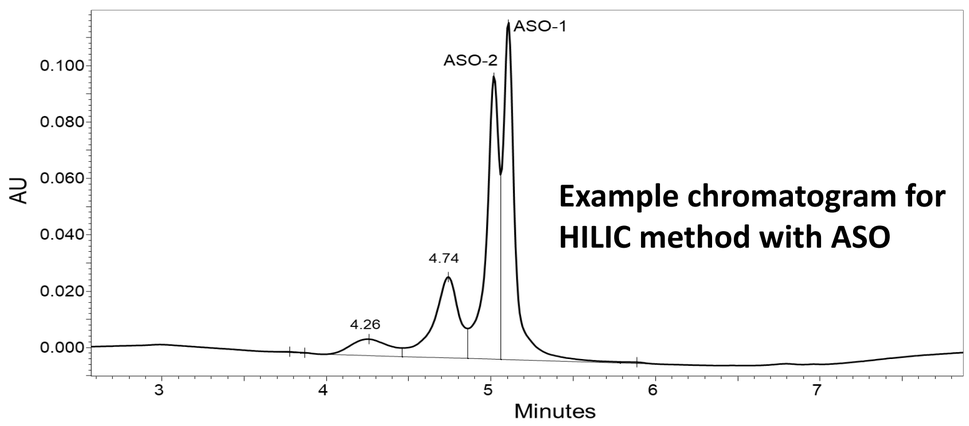
  MOE = methoxyethyl 2' modification, MeC = 5-methyl cytidine residue

  5' palmitate modification

Analytical Method

- Loss of active (% purity) monitored using hydrophilic interaction liquid chromatography (HILIC).
  - Hydrophilic (amide) stationary phase packed into MaxPeak™ High Performance Surfaces (HPS): reduces nonspecific binding, eliminates need for column passivation, and increases reproducibility.
  - Oligonucleotides strongly retained at high organic conditions, with retention influenced by strand length, column temperature, and mobile phase ionic strength/aqueous content.

Mobile Phase	**A**: 90:10 ACN:100 mM NH_4OAc **B**: 10:90 ACN:100 mM NH_4OAc		
Gradient	**Time (min)**	**% A**	**% B**
	0	90	10
	0.67	90	10
	4.67	22	78
	5.00	22	78
	5.33	90	10
	13.33	90	10
Flow Rate	0.3 mL/min		
Column Temp.	75°C		
Injection Vol.	10 µL		

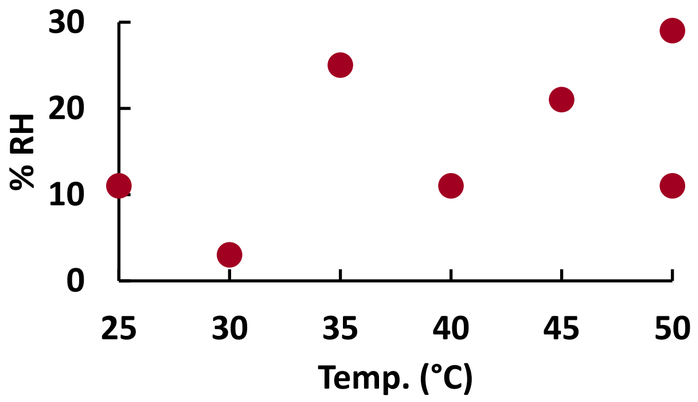


Example chromatogram for HILIC method with ASO

ASAP Study Design

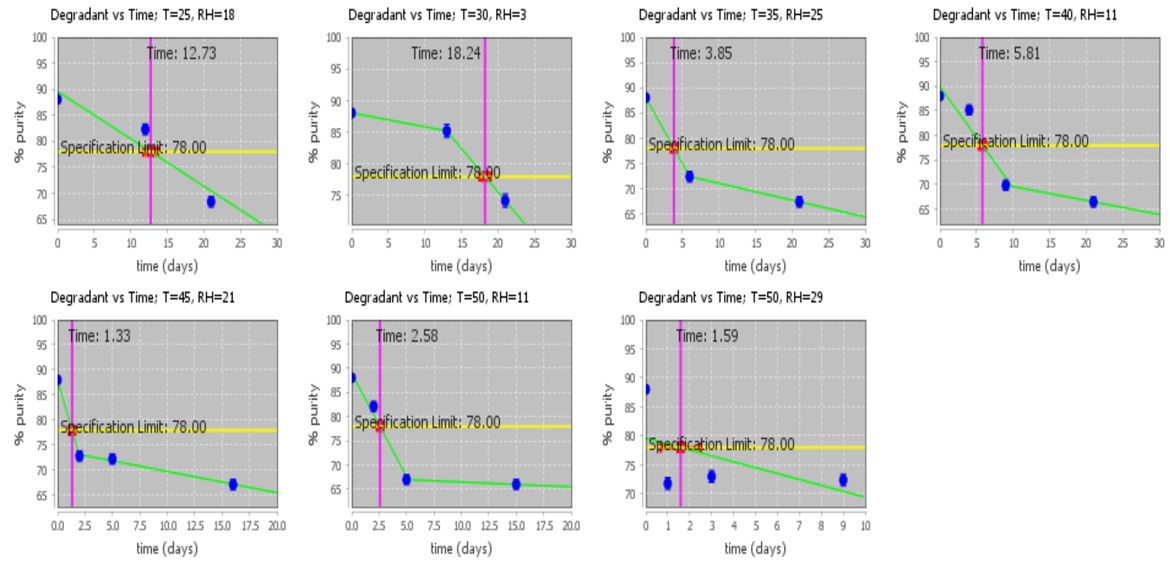
- Experimental design aims to bracket specification limit (10% loss of active).
- Samples exposed, open, to temperature/relative humidity conditions.

Temp (°C)	Saturated Salt	% RH	Time (d)
Control	NA	NA	0 (n = 3)
25	LiCl	11	12
30	CsF	3	13, 21
35	KF	25	6, 21
40	LiCl	11	4, 9, 21
45	KF	21	2, 5, 16
50	LiCl	11	2, 5, 15
50	NaI	29	1



Results

- Non-linear behavior observed (two-step process).
- Isoconversion times (time to lose 10% purity) determined using bilinear fitting.



- Isoconversion rates (k) well fit by moisture-modified Arrhenius equation:

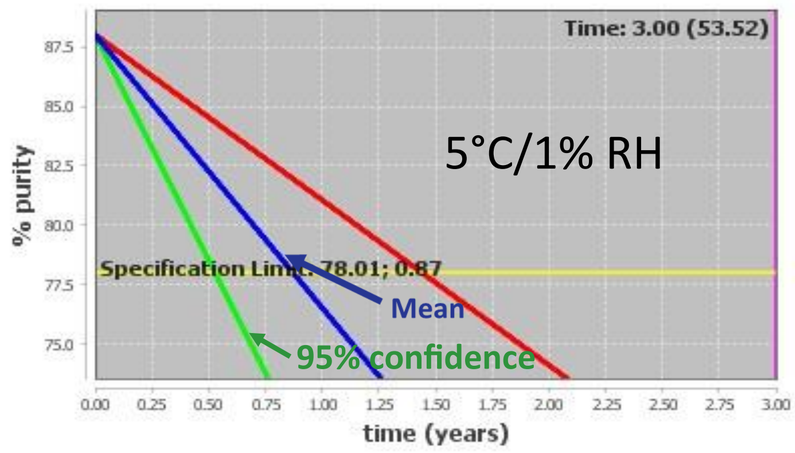
$$\ln(k) = lnA - \frac{E_a}{RT} + B(RH)$$

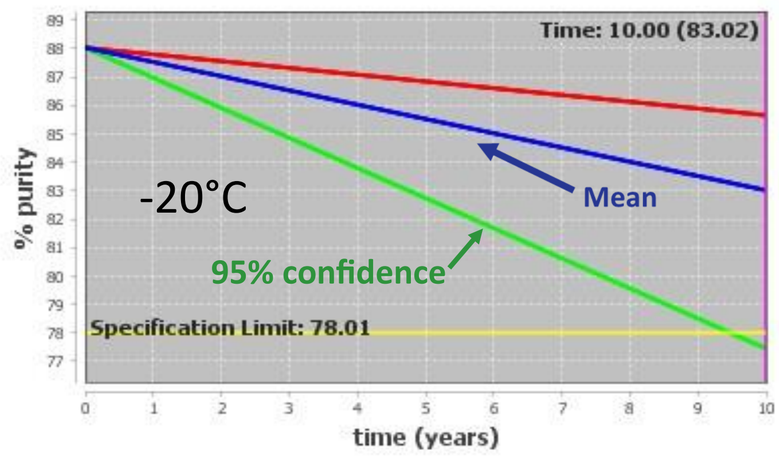
- ln A = intercept, E_a = activation energy [temp sensitivity], R = gas constant, T = temp in K, B = humidity sensitivity, RH = equilibrium humidity

lnA	E_a (kcal/mol)	B	R^2	Q^2
27.5 ± 3.7	17.2 ± 2.2	0.066 ± 0.011	**0.982**	**0.945**

ASAP Model Predictions

- ASAP shelf life modeling (using ASAP*prime*®) to 95% probability of passing:
  - Less than six months at 5°C, even very dry (with molecular sieves).
  - More than nine years at -20°C.





Conclusions

- Three-week ASAP study conducted on lyophilized lipid-conjugated ASO.
  - HILIC method provides effective separation.
- Loss of purity well fit by moisture-modified Arrhenius equation using isoconversion.
  - Lipid-conjugated ASO requires frozen storage (-20°C) for long-term stability.
- Stability of RNA-based molecules is effectively modeled using ASAP.

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