

Analysis of RNAi and RNAi duplexes with UPLC-MS

Martin Gilar, Sean M. McCarthy and Vera Ivleva
Waters Corporation, 34 Maple Street, Milford, MA 01757

OVERVIEW

- Method development for fast oligonucleotide separation with Ultra Performance Liquid Chromatography (UPLC) is discussed.
- 1-2 minute separation of 21nt RNAi with N-1 resolution.
- RNAi characterization with LC-MS.
- Confirmatory MS/MS sequencing of 21nt RNAi.
- Non-denaturing mobile phases for LC-MS duplex RNA analysis.
- Resolution of duplexes from single stranded RNAi oligos.

FAST UPLC OLIGONUCLEOTIDE SEPARATIONS

- Use 2.1 x 50 mm, 1.7 μ m ACQUITY OST C₁₈ column with 0.2 mL/min mobile phase flow rate (FR) at 60 °C.
- Select an appropriate initial mobile phase strength (% of MeCN) and gradient slope to achieve a desirable resolution.
- Increase FR while decreasing gradient time proportionally (Figure 1). Gradient volume remains constant, analysis time decreases with minor loss of resolution. Resolution of RNAi from the n-x sequences within 1-2 minutes is possible (Figure 2).

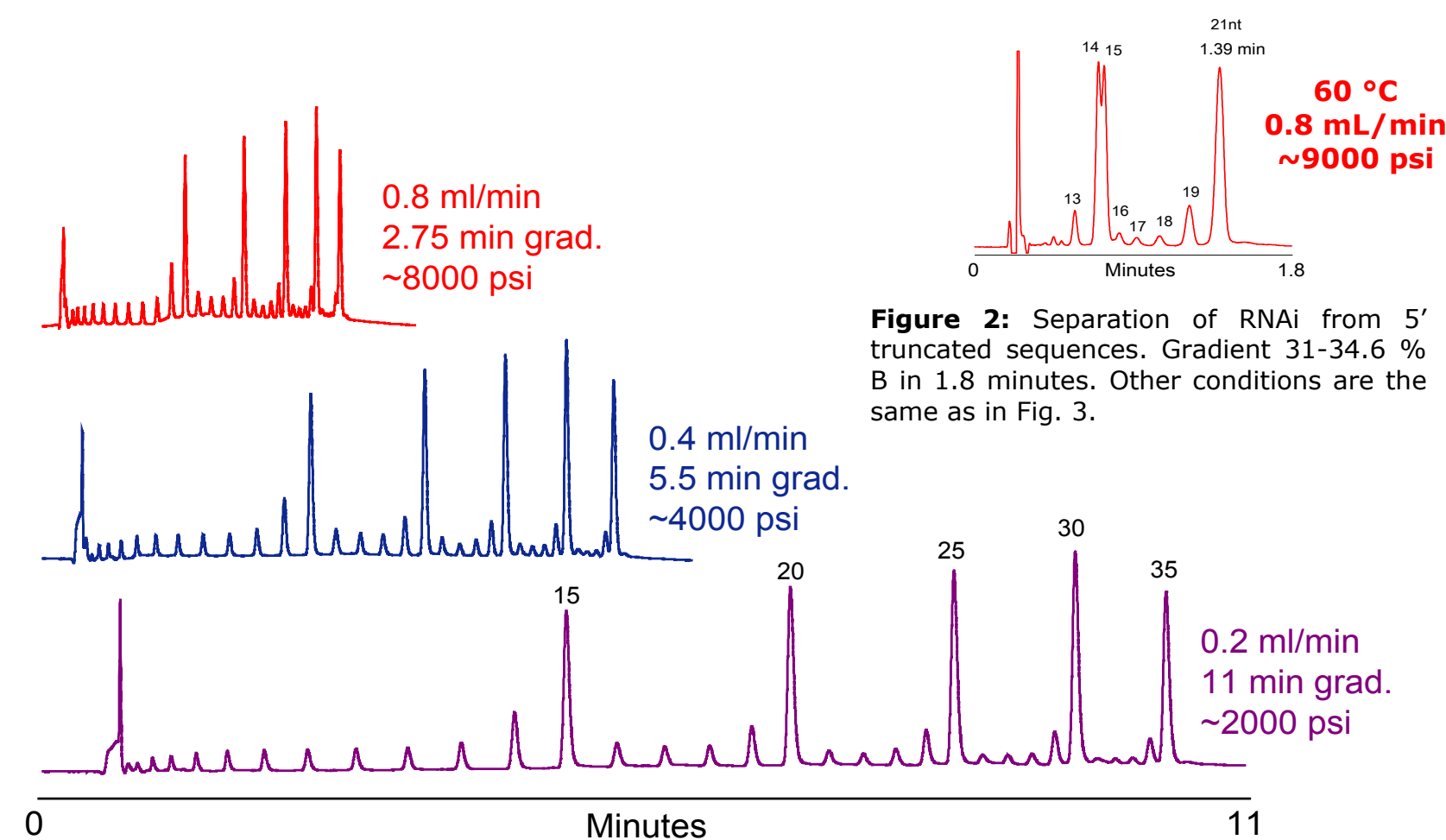


Figure 1: Separation of 2-35nt oligodeoxythymidine standard (PN 186004135) Waters ACQUITY UPLC® System and ACQUITY UPLC® OST C₁₈, 1.7 μ m, 2.1x50 mm column, 60 °C, UV 260 nm. Mobile phase A: 100 mM Hexylammonium acetate, pH 7, B: 50% m.p. A, 50% MeCN. Gradient was 56-80.2% B for all separations.

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

RNAi CHARACTERIZATION WITH LC-MS

- Use LC-MS compatible mobile phases, such as 15mM TEA, 400 mM HFIP ion-pairing buffer.
- Sum mass spectra under the peaks of interest, deconvolute mass with MaxEnt1, calculate Δ mass between adjacent peaks, assign failed sequences based on Δ mass (Figure 3).
- MS/MS sequencing of 21nt RNA was performed with Synapt MS (Figure 4).

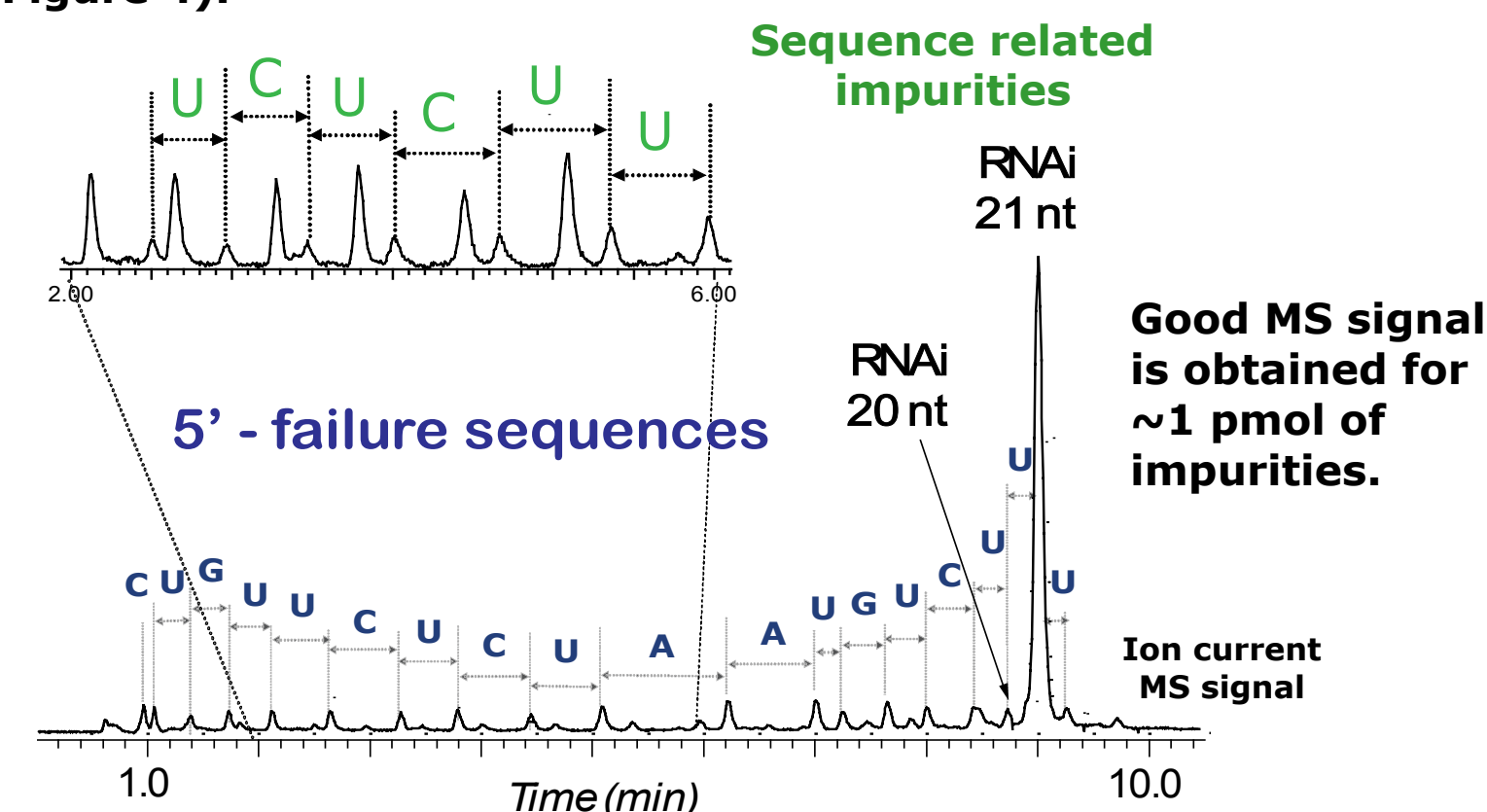


Figure 3: UPLC analysis of crude 21nt RNAi, UUC UGU AAU CUC UUG UCU ATT (5'-3'), ACQUITY UPLC® OST C₁₈, 1.7 μ m, 2.1x50 mm column, 60 °C, UV 260 nm. M.p. A: 15mM TEA, 400 mM HFIP, pH 7.9, B: 50% m.p. A, 50% MeOH. Gradient 20-40 % B in 10 minutes, 0.2 mL/min.

