# The Effect of Gas-Phase Reactions on the Quantitation of Cyclic Hydrazone Libraries by Electrospray Ionization (ESI) Mass Spec

**Holly Schiltz,[a] Mee-Kyung Chung[a], Stephen J. Lee[b] and Michel R. Gagné*[a]**

## Supplemental Information

### HPLC-LP Analysis

HPLC analysis was performed on a Hewlett-Packard Series 1100 instrument, using an Agilent Eclipse XDB-C18 column (4.6 x 150 mm, 4.6 µm) with gradient elution (methanol/acetonitrile/water) at a flow rate of 1.0 mL/min (column temperature: R.T.). The injection volume for a 5 mM DCL was typically 10 µL. UV absorbance chromatograms were recorded at wavelengths of 220 and 289 nm. The laser polarimeter signal was recorded with a PDR-Chiral, Inc. Advanced Laser Polarimeter equipped with a 3.5 mW laser diode system (670 nm) and an 18 µL flow cell. The data was analyzed using the HP Chemstation software.

To remove the solvent, TFA, and the template, 10% methanol/water for 3 min and then a linear gradient (10 to 40% methanol/water over 6 min) was used. A second gradient (10% acetonitrile/water for 5 min then increased to 43% acetonitrile/water over 17 min and steady 43% acetonitrile/water for 2 min) was subsequently used to separate the library. The convoluted procedure removed traces of solvent and the template which adversely affected the LP baseline.

### UPLC-MS Analysis

UPLC-MS analysis was performed on Waters Acquity UPLC$^{TM}$ instrument, using an Acquity UPLC® BEH C18 column (2.1 x 100 mm, 1.7 µm) with various gradient elution (17 to 30% acetonitrile/water containing 0.2% formic acid, in 12.5 min) either at a flow rate of 0.3 mL/min (column temperature: 29 °C) or 0.7 mL/min (column temperature: 55 °C). The injection volume for a 5 mM DCL was typically 1.5 µL. The eluent was analyzed by a Waters Micromass ZQ mass spectrometer in positive ion mode with the electrospray ionization source. Mass chromatogram conditions were optimized: 2.7 kV capillary voltage, 50 V cone voltage, 150 °C source temperature, 350 °C desolvation gas temperature and 250 L/h desolvation gas ($N_2$) flow rate. Data were collected and analyzed using the MassLynx software.

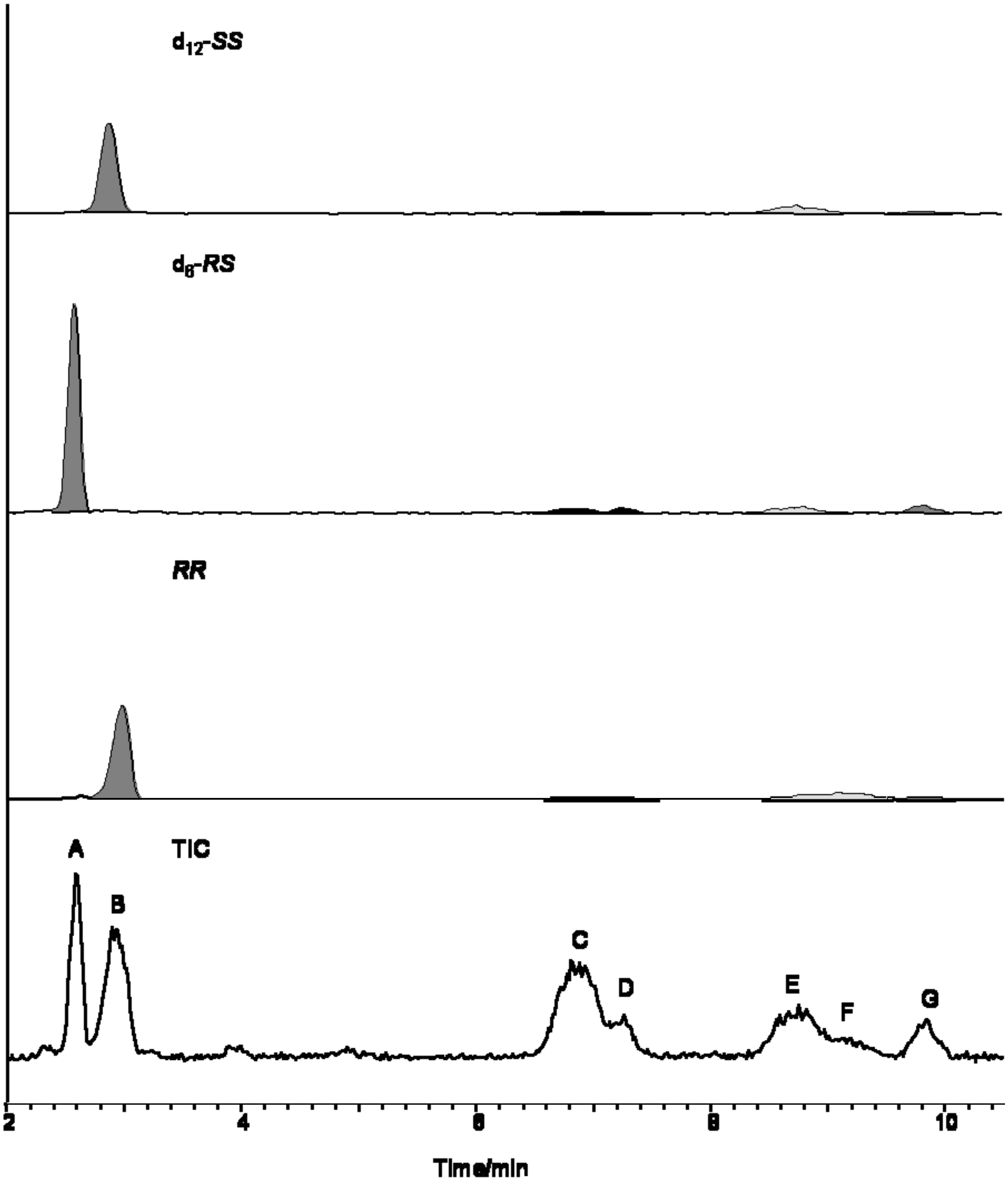

**Fig. 1** Selected mass ion chromatograms for all three species of dimer in the DCL. The first three traces are shown in correct proportions and normalized to the $d_6$-RS mass range, which is most populated. The TIC is not to scale in reference to the dimer traces, but serves as a reference to the regions of species.

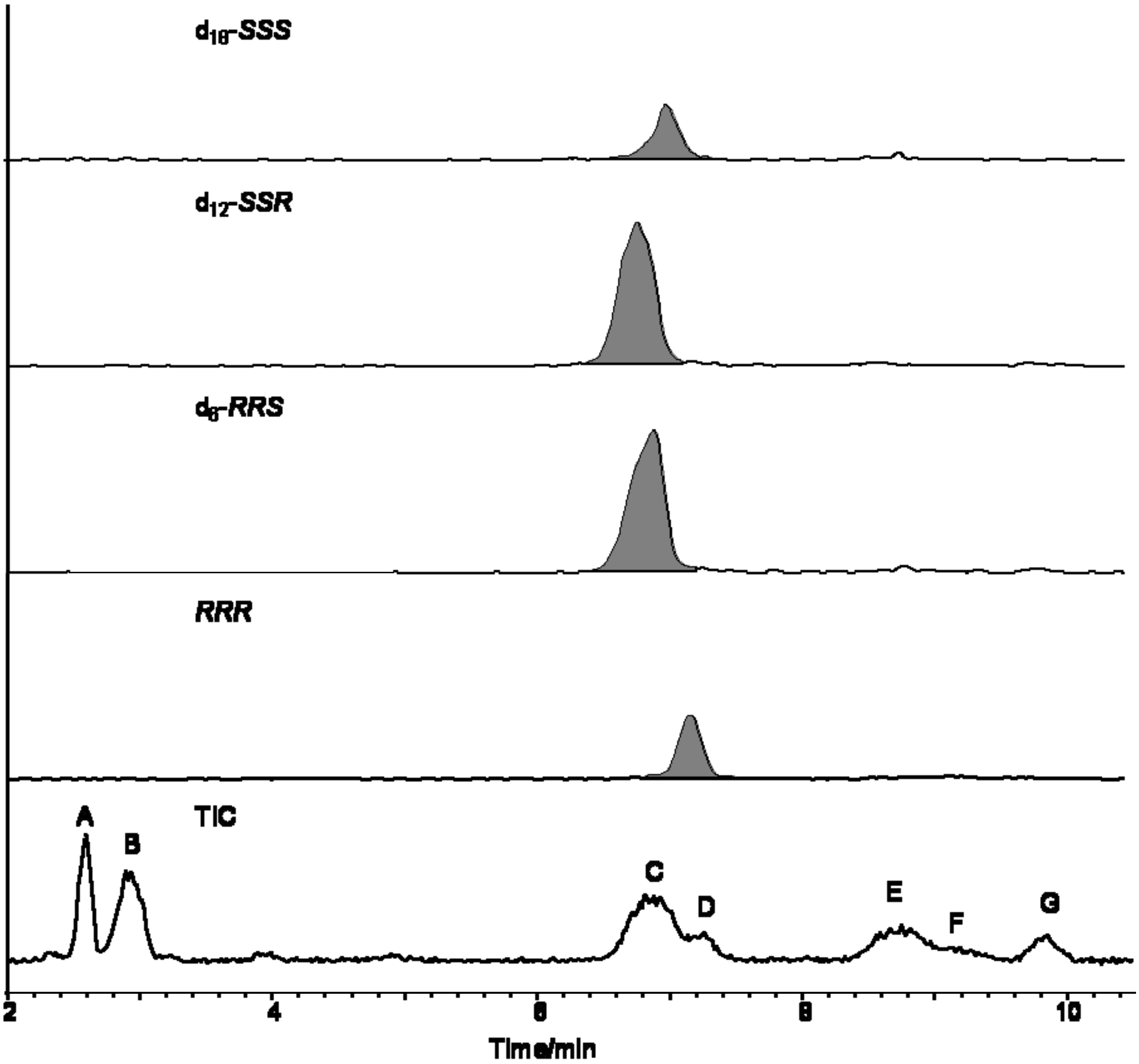

**Fig. 2** Selected mass ion chromatograms for all 4 species of trimer in the DCL followed by the reference TIC. All trimer traces are normalized to the $d_{12}$-SSR mass range. The retention times of the RRR and SSS species reflect the fragmentation of these species to dimer in Fig. 1 and the oligomerization to meso-tetramer in Fig. 3.

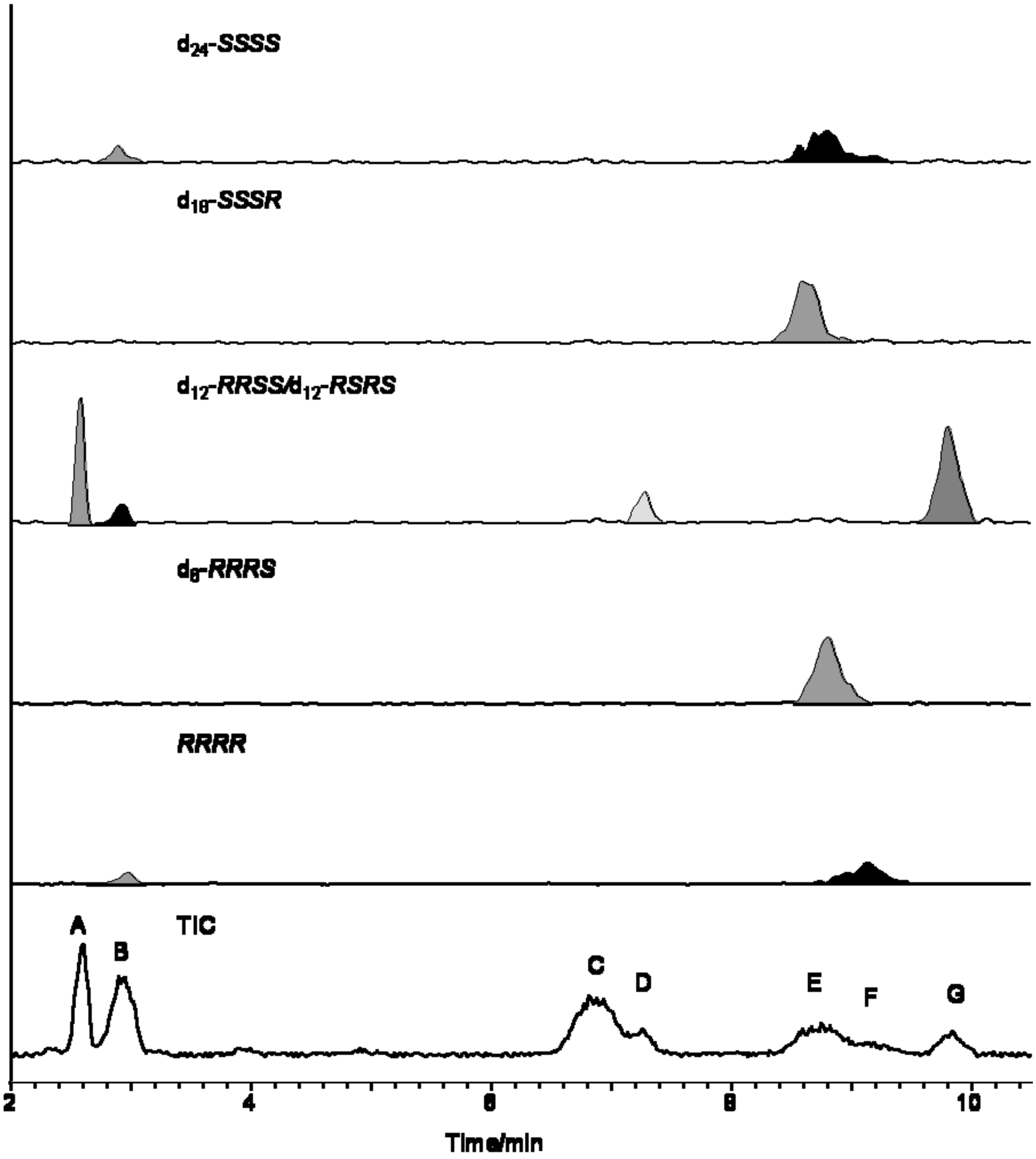

**Fig. 3** Selected mass ion chromatograms for all five mass ranges associated with the six tetramer species in the DCL. The TIC at the bottom provides a regional reference to the preceding traces. All tetramer chromatograms are normalized to the $d_{12}$-*RRSS*/$d_{12}$-*RSRS* trace.

## 5 Notes

[a] *Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA 27599-3290. E-mail: mgagne@unc.edu*
[b] *U.S. Army Research Office, P.O. Box 12211, Research Triangle Park, North Carolina, USA 27709. E-mail: Stephen.Lee2@us.army.mil*