1 Supplementary Materials

2 Modifying Protocol for HPLC-MS/MS

3 The UHPLC-MS/MS used in the assay we described may not be available to all researchers

4 interested in this method. Tandem mass spectrometers have become widely available at many

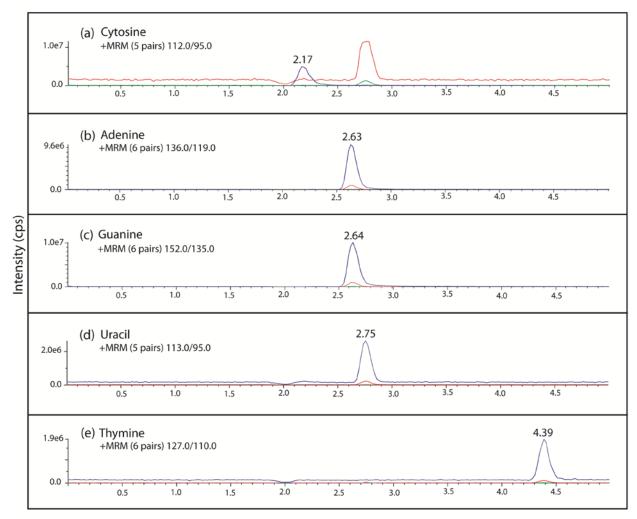
- 5 research institutions, while UHPLC equipment is less common. Built-in converter software
- 6 applications are readily available to convert UHPLC methods to conventional HPLC methods,
- 7 and vice versa, with concise instructions provided to the users. Such software and instructions
- 8 are routinely available from the LC or MS vendors on request. Further, a literature search will
- 9 reveal a number of publications on the topic of migrating UHPLC to HPLC and vice versa, such
- 10 as Novakova et al. (2011). We selected UHPLC for the described assay, because it provides
- 11 significant reduction in analysis time without loss in peak resolution.

Nováková L, Veuthey JL, Guillarme D. 2011. Practical method transfer from high
performance liquid chromatography to ultra-high performance liquid chromatography: The
importance of frictional heating. J Chromatogr A. 1218: 7971–7981

Figure S1: Chromatograms of each nucleobase showing the elution order of a 1:1:1:1:1 mixture (1.0 ng each) of commercially supplied (a) cytosine, (b) adenine, (c) guanine, (d) uracil and (e) thymine. The blue line corresponds to the MRM trace of the ¹²C species of each nucleobase, while red corresponds to the MRM_{C12+1}. The cross-talk of signals from uracil into the cytosine channel (a, red and green lines) is due to the overlapping m/z of fragments.

- **Figure S2**: Quantitation of ¹³C-enriched RNA in CsTFA gradient fractions as a proportion of
- total RNA recovered (A) and by degree of enrichment (B). N.A. refers to the average % atom 13 C
- 22 in unlabeled RNA.

Figure S1



Time (min)



