|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **1 minute UPLC Gradient** | | | | |
| **Column:** | Waters Acquity UPLC BEH C18 1.7µm 2.1x30mm | | | |
| **Mobile Phase A:** | Water with 0.1% Formic Acid | | | |
| **Mobile Phase B:** | Acetonitrile with 0.1% Formic Acid | | | |
| **Wash Solvent 1:** | 20:80 Methanol/Water | | | |
| **Wash Solvent 2:** | 50:50 Isopropanol/Acetonitrile | | | |
| **Temperature (°C):** | 60 | | | |
| **Step** | **Total Time (min)** | **Flow Rate (µl/min)** | **A1 (%)** | **B1 (%)** |
| 0 | 0.00 | 1000 | 99 | 1 |
| 1 | 0.05 | 1000 | 99 | 1 |
| 2 | 0.80 | 1000 | 1 | 99 |
| 3 | 0.90 | 1000 | 1 | 99 |
| 4 | 0.91 | 1000 | 99 | 1 |
| 5 | 1.00 | 1000 | 99 | 1 |
| **2 minute UPLC Gradient** | | | | |
| **Column:** | Waters Acquity UPLC BEH C18 1.7µm 2.1x50mm | | | |
| **Mobile Phase A:** | Water with 0.1% Formic Acid | | | |
| **Mobile Phase B:** | Acetonitrile with 0.1% Formic Acid | | | |
| **Wash Solvent 1:** | 20:80 Methanol/Water | | | |
| **Wash Solvent 2:** | 50:50 Isopropanol/Acetonitrile | | | |
| **Temperature (°C):** | 40 | | | |
| **Step** | **Total Time (min)** | **Flow Rate (µl/min)** | **A1 (%)** | **B1 (%)** |
| 0 | 0.00 | 500 | 95 | 5 |
| 1 | 0.10 | 500 | 95 | 5 |
| 2 | 1.60 | 500 | 5 | 95 |
| 3 | 1.75 | 500 | 5 | 95 |
| 4 | 1.76 | 500 | 95 | 5 |
| 5 | 2.00 | 500 | 95 | 5 |

Table S3. Summary of UPLC conditions used for the analysis of standard *in vitro* DMPK assays. The two gradients methods are available on each instrument in the lab, and the method used is determined based on the assay.