**UHPLC-MS/MS method with online SPE for thalidomide quantification in dried plasma spot for therapeutic drug monitoring**

**SUPPLEMENTARY DATA**

Table S1 – Mass spectrometry parameters and typical retention times for thalidomide and its stable-isotope-labeled internal standard

| Compound | Retention time (min) | Precursor ion (m/z) | \*Product ion (m/z) | Collision energy (eV) | Tube Lens Voltage (V) | Target Ion ratio |
| --- | --- | --- | --- | --- | --- | --- |
| thalidomide | 5.7 | 259.1 | 84.1 | 13 | 70 |  |
|  |  |  | 186 | 17 | 70 | 87.2 |
| thalidomide-d4 | 5.7 | 263.1 | 84.1 | 13 | 70 |  |
|  |  |  | 190 | 17 | 70 | 76.3 |

ESI: Electrospray ionization; m/z: mass/charge ratio; \*Product ions are given as quantifying ion; confirming ion; eV: electron Volts; V: Volts.

Table S2 – Online solid phase extraction (SPE) clean up elution program

| Time | Flow rate | Solvent A | Solvent B |
| --- | --- | --- | --- |
| min | mL/min | % | % |
| Initial condition | 1.000 | 100.0 | 0.0 |
| 0.0 | 1.000 | 100.0 | 0.0 |
| 1.0 | 1.000 | 100.0 | 0.0 |
| 3.0 | 1.000 | 80.0 | 20.0 |
| 3.1 | 1.000 | 15.0 | 85.0 |
| 6.9 | 1.000 | 15.0 | 85.0 |
| 7.0 | 1.000 | 100.0 | 0.0 |
| 8.0 | Stop run | | |

Optimized SPE online clean up with SolExTM RSLC HRP column, 2.1x20mm, set at 40ºC and eluted with a mixture of organic and aqueous phases. Solvent A: 0.5% formic acid in water; Solvent B: 0.5% formic acid in 50% of methanol, 40% of acetonitrile and 10% of water.

Table S3 – Gradient elution program

| Time | Flow rate | Solvent A | Solvent B |
| --- | --- | --- | --- |
| min | mL/min | % | % |
| Initial condition | 0.400 | 73.0 | 27.0 |
| 0.0 | 0.400 | 73.0 | 27.0 |
| 3.0 | 0.400 | 73.0 | 27.0 |
| 5.0 | 0.400 | 10.0 | 90.0 |
| 6.5 | 0.400 | 10.0 | 90.0 |
| 6.6 | 0.400 | 73.0 | 27.0 |
| 8.0 | Stop run | | |

Optimized chromatographic method 50X 2.1mmX 2.6µm AccucoreTM PFP. coupled with a guard column 2.1x20 mm (Security Guard Cartridge C18 Phenomenex, Torrance, CA, USA). set at 40ºC and eluted with a mixture of organic and aqueous phases. Solvent A: 0.1% formic acid in water; Solvent B: 0.1% formic acid in 70% of methanol and 30% of acetonitrile. Volume injected 60 µL.