Supplemental Digital Content 4

Doxorubicin quantification protocol

Sample collection

- Collected weighted blood and tissue (~100 mg) samples in 2 mL eppendorf cups

Calibration line

- Prepared a calibration line from 10, 20, 30, 40, 50 µL of the 20 x diluted TSL injectate

Homogenisation

- Added 1.5mL daunorubicin in H_2O (2 µg/mL daunorubicin) to each sample
- Homogenisation with Tissuelyser (Qiagen) using stainless steel beads (Qiagen, 5mm) for 30 - 60 min at 30Hz

Extraction

- Transfered 125 µL sample to preweighed 2 mL eppendorf cups and stored at -80 °C until further processing
- Added 50 µL 1.94 M silver nitrate solution & vortex
- 10 min. incubation at 20°C
- Added 1.25 mL chloroform/isopropanol (2:1 *v/v*) and vortexed for 5 minutes
- 10 min of centrifugation at 1200 x g at 20°C
- Transfer the organic (lower) phase to glass tube (Duran, 12 x 75 mm)
- Evaporated the organic solvents at 40°C under N_2-flow
- Dissolved in 200 µL MilliQ water and transferred sample to HPLC vial

HPLC analysis

- HPLC analysis was performed on an Agilent Technologies system (1100 series) equipped with an autosampler and fluorescence detector (λex 485 nm and λem 590 nm). 50 µL of each sample was injected on an Eclipse XDB-C18 column (5 mm, 4.6 ? 150 mm2 Agilent). The doxorubicin and daunorubicin were eluted in 6 and 12 min
  respectively, using an isocratic flow of 1 mL/min with 30% (v/v) acetonitrile in H2O containing 0.1% TFA (v/v).