Barratt DT, Cox HK, Menelaou A, Yeung DT, White DL, Hughes TP, Somogyi AA. CYP2C8 genotype significantly alters imatinib metabolism in chronic myeloid leukaemia patients. Clinical Pharmacokinetics.

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Electronic Supplementary Material 2: Determination of plasma imatinib and N-desmethyl imatinib concentrations in chronic myeloid leukaemia patients

Imatinib and N-desmethyl imatinib plasma concentrations were determined using HPLC with UV detection at 267 nm. The mobile phase consisted of 30% acetonitrile and 70% KH$_2$PO$_4$ (20 mM, final concentration) and run at a flow rate of 1.0 ml/min. Plasma (100 µL) was aliquoted into microcentrifuge tubes. Plasma proteins were then precipitated by the addition of methanol (150 µL) followed by vortexing (2 min) and storage at -20°C (60 min). Samples were then centrifuged (15 min; 14000 rpm) and the supernatant (50 µL) injected on to a reverse phase HPLC column (Kromasil C8, 5 µm, 250 x 4.6 mm ID, Grace Alltech, Deerfield, IL, USA) housed within a column oven set to 40°C. Total run time per sample was 15 min. Calibration standards for both analytes of interest ranged from 100 to 25000 ng/ml and high (7500 ng/ml), medium (2000 ng/ml) and low (300 ng/ml) quality control (QC) samples were assayed along with each set of plasma samples.

The assay was validated and found to be precise, accurate and suitable for therapeutic drug monitoring. The assay was also cross-validated and certified as part of the ‘Imatinib (Glivec) Blood Level Testing Program’ run by TDM Pharmaceutical Research, LLC, Newark, DE, USA. Intra-assay (n = 6) and inter-assay (n = 4) precision (coefficient of variation) and inaccuracies were less than ± 10% of nominal concentrations for all QC samples and less than ± 20% of nominal concentration for the lower limit of quantification (LLOQ, 100 ng/ml), for both analytes of interest.