Supplementary Data

Method validation procedure and acceptance criteria.

Parameter	Procedure	Acceptance criteria
Selectivity	Ten human plasma lots of plain plasma containing dipotassium EDTA as an anticoagulant were processed along with their respective LLOQ samples and analysed for selectivity exercise. The percent interference was calculated for endogenous components present in plasma at the retention times of the analyte and the IS. *Percent Interference = 100 x [Interference of peak response (analyte and ISTD)/ Average area of six LLOQ samples]*	The interfering peaks at the retention time of the analyte must be < 20% of the respective plasma blanks extracted mean LLOQ peak area. Response of interfering peaks at the retention time of internal standard must be < 5% of the respective mean response of internal standard in LLOQ sample. At least 80% of the blank screened matrix lots should be meets the above acceptance criteria.
Recovery	Prepared 'post extracted samples' and samples of empagliflozin and metformin LQC, MQC and HQC levels in six different plasma lots of plain plasma and termed as 'extracted samples'. The area response of analyte and internal standard in 'extracted samples' were compared against area response of analyte and internal standard in 'Post extracted sample' in each of 6 lots of plain plasma *Percent recovery = 100 x (Mean Analyte peak response in extracted samples/ Mean Analyte peak response in unextracted (post spiked samples) samples]*	The percent recovery of the analyte and the internal standard should not be more than 115%. The CV for the % recovery of analyte across LQC, MQC and HQC levels should be ≤ 15%.
Matrix effect	Prepared recovery comparison samples at LQC, MQC and HQC level containing internal standard dilution representing 100% extraction and termed as 'recovery comparison solution'. Three sets of blank plasma samples (i.e. six batches of plain blank plasma containing dipotassium EDTA as an anticoagulant) were extracted up to the stage of evaporation and	The percent response ratio at LQC and HQC level should be within 85-115%.

	were reconstituted with LQC, MQC and HQC recovery comparison solutions and termed as 'Post extracted samples'. The area response of analyte and internal standard in 'Post extracted samples' was compared against the 'recovery comparison solution' to evaluate matrix effect. % Response ratio = 100 x (Mean area ratio of post spiked samples/ Mean area ratio of equivalent aqueous samples)	
Accuracy and precision	The Accuracy & Precision batches are organized in the following manner: 1. Reconstitution solution / Mobile Phase x 1 2. Standard blank (without Analyte, Internal Standard) x 1 3. Standard zero (with internal standard) x 1 4. 6 – 8 non-zero CC standards (LLOQ and ULOQ) 5. LLOQ QC, LQC, MQC, HQC A total of 3 (three) Accuracy & Precision batches were run. The Precision of the assay was measured as the percent coefficient of variation over the concentration range of LLOQ QC, LQC, MQC-A, MQC and HQC samples. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of LLOQ QC, LQC, MQC-A, MQC and HQC samples to their respective nominal values, expressed in percentage.	*Accuracy:* The within and between batch mean value should not deviate by more than 15% of the nominal value at low, medium and high QC concentrations except LLOQ QC where it should not be more than 20%. *Precision:* The within and between batch %CVs for low, medium and high concentrations should be within 15% except LLOQ QC for which %CV should not exceed by more than 20%.
Stabilities	All stabilities of empagliflozin and metformin from biological matrix were carried out by quantifying six sets each of LQC and HQC samples against freshly spiked calibration curve standards and six sets each of LQC and HQC (freshly removed and processed) samples to validate the run. For each stability, %stability was calculated as % accuracy of stability quality control samples with respect to their nominal concentration. For autosampler stability and bench top stability only those samples were considered which were processed and injected together for calculation of internal standard variation.	

	% Stability = 100 X (Mean concentration of stability samples/ Mean concentration of comparison samples)	
Autosampler	In assessing the autosampler stability, six sets of quality control samples (LQC and HQC) of bulk spiking check stored in the autosampler, were reinjected after 54 hrs for empagliflozin and metformin.	The percent stability of stability samples should be within 85-115%.
Bench top	Bench top stability, using six sets each of LQC and HQC samples, was determined by keeping quality control samples on bench top for 20 hrs for empagliflozin, and metformin.	The percent stability of stability samples should be within 85-115%.
Freeze-thaw	The stability of empagliflozin and metformin from human plasma was determined for six freeze-thaw cycles. Six replicates of LQC and HQC were analysed for six freeze-thaw cycles. The freeze-thaw stability quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for the calculation of precision and accuracy	The percent stability of stability samples should be within 85-115%.