After 24 hours of leaving the samples in the autosampler the same three samples for each (QC low and QC high) were injected to be analyzed again and the resultant concentrations were also calculated.

2.2.3.5 Recovery

Drug and IS recovery

Two groups of QC low, mid and high were prepared. In the first group both the drug and the IS were prepared in the mobile phase while the second group was spiked in serum or Krebs buffer. CV% values were calculated from the resultant AUC of both the drug and the IS followed by absolute recovery calculation for each of them.

2.2.4 Animal handling

The protocols for the animal study were approved by the Ethical Committee of the Higher Research Council at the Faculty of Pharmacy and Medical Sciences, University of Petra (Amman, Jordan). Adult males and non-pregnant females Sprague Dawley rats were supplied and housed at the University of Petra's Animal House. Rats with average weight of (220 ± 20 g) were used. Rats were kept in air-conditioned environment under controlled temperatures (22-24 °C), humidity (55-65%), and photoperiod cycles (12 light/12 h dark). Rats were fasted overnight (for 18 to 22 h) with free access to water, unless otherwise stated. All experiments were achieved in accordance with University of Petra's Institutional Guidelines on Animal Use which adopts the guidelines of the Federation of European Laboratory Animal Science Association (FELASA).