Development of a LC-MS/MS method for quantitation of 5FU from Telimmune® cards for the purpose of therapeutic drug monitoring

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Background: Studies have shown improvement in overall survival of colorectal cancer patients receiving infusional 5 fluorouracil (5FU) when target area under the time concentration, measured using a steady-state plasma 5FU concentration, is maintained between 20 -30 mg*h/L. Currently, optimal sampling for therapeutic drug monitoring (TDM) is obtaining plasma from a venous blood sample 18h or later after commencing the infusion. However, this requires patients to attend a clinic for venipuncture collection, and the sample must then be transported to the laboratory. This is inconvenient for patients and may require travelling long distances to a suitable facility, limiting access to TDM for patients living in remote areas. As a possible alternative, we are investigating the Telimmune® card microsampling device which produces a dry plasma spot from capillary whole blood, for use in 5FU measurement, enabling self- sampling by a patient and facilitating 5FU TDM.
Aims: to develop and validate a LC-MS/MS method for measurement of 5FU from a dry plasma spot obtained using the Telimmune cards®.
Methods: Validation was performed according to the ICH guidelines in terms of linearity, precision and accuracy, specificity, and stability. Fresh whole blood spiked with 5FU solution was used for preparation of calibrators and quality controls and applied onto the Telimmune cards®. After 3 minutes, top layer of the cards was peeled off leaving the plasma collection discs to air dry for at least 15 minutes before extraction. Samples were extracted using acetonitrile:water (80/20 v/v) mixture and chromatographically separated on a Luna Omega Polar C18 column. Mobile phases consisted of water and acetonitrile with isocratic flow at 0.5 ml/min over 3 minutes.
Results: Assay was linear across the concentration range of 0.05-5 mg/L. Accuracy ranged from 102 to 111 % and maximum imprecision was < 10 %. Stability was demonstrated at room temperature and 50°C for up to 5 weeks and 3 days, respectively (degradation < 9 % at 3 concentrations).
Discussion: Having validated the method for quantification of 5FU from Telimmune cards®, the next planned step is a study assessing the relationship between venous and capillary plasma obtained from cancer patients undergoing treatment with 5FU.