Peptide Analysis Using Dry Blood Spots Combined with Mass Spectrometry: A Practical Approach for Minimizing Animal Use

W. Lee, J. Chapdelaine, J. Tremblay, A. Nakai, D. Poulin, W. Ruddock & G. Bain
ITR Laboratories Canada Inc., Montreal, QC, Canada

Objective:

To demonstrate the applicability of using 10 mcL volume dry blood spots combined with mass spectrometric detection for toxicokinetic analysis of a large peptide in mice.

Introduction:

The interest for analysis of therapeutic peptides for toxicokinetic and pharmacokinetic studies is increasing as more companies are striving to find new chemical entities. Biologicals (includes peptides and proteins) now account for 30% of all drugs in research and development, therefore creating a need for increased efficiency and reliability of TK and PK data generation. One approach for this is to combine the small volume requirements of dry blood spot sample collection with the sensitivity, selectivity and reliability of mass spectrometric detection.

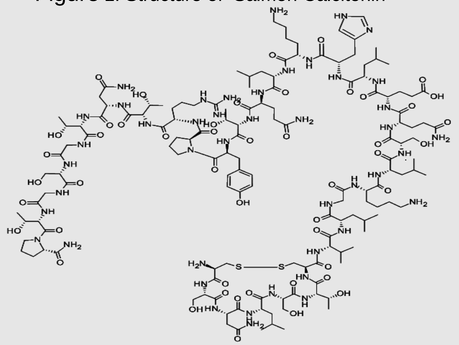
Using dry blood spotting allows for collection of 5 to 50 mcL amounts of blood per timepoint that can then be used for determining systemic exposure, bioavailability or dose proportionality in toxicokinetic studies. By keeping the blood draw volume low, a full TK profile is feasible on each small rodent that is studied. This allows application of the 3R principles, that is to reduce, refine and replace for preclinical studies.

Methods:

The peptide salmon calcitonin (indicated for the treatment of postmenopausal osteoporosis) was chosen based on it being readily available from Anaspec and that its molecular weight is higher than typically quantified by mass spectrometry. Salmon calcitonin consists of 32 amino acids and has a molecular weight of over 3.4 kDa. Human calcitonin, was chosen as internal standard and was also purchased from Anapsec.

For the in-vivo work, a dose formulation solution containing 1 mg/mL of salmon calcitonin in water was injected subcutaneously into a 26.6 gram CD-1 mouse. The total dose administered was 10 mg/kg, that was 270 mcL of the 1 mg/mL dose formulation solution. Using a tail clipping technique, 10 mcL of blood was then collected from pre-dose until 120 minutes using Microcaps micro capillary tubes purchased from Drummond Scientific. The collected blood was then spotted onto Whatman 903 Protein Saver cards that were dried at room temperature before storing with desiccant.

Figure 1: Structure of Salmon Calcitonin



Molecular Formula: $C_{145}H_{240}N_{44}O_{48}S_2$
Formula Weight: 3431.9 amu

The extraction procedure was developed at ITR Laboratories Canada. Calibration standards and quality control samples ranged from 20.0 to 2000 ng/mL in concentration (equivalent to 0.200 to 20.0 ng per blood spot). On the day of analysis, spots of calibration standards, quality control samples, and the mouse samples were punched out using a 5/16 inch hole puncher to removed the entire spot, ensuring the full 10 mcL blood volume was extracted. The spots were soaked in diluent, vortexed and then sonicated. The extract was filtered before injection for HPLC mass spectrometric analysis.

The injection parameters used were developed at ITR Laboratories Canada. Chromatography was developed on a Waters Xbridge® C18 column with dimensions of 50 x 4.6 mm, with 3.5 micron packing. The mass spectrometric conditions were optimized to obtain a +4 charge for the parent ion (859 mass to charge) and this was fragmented to the main product ion (1107 mass to charge). The MRM transition of 859 to 1107 provided a selective transition due to the higher fragment ion versus parent ion that is uncommon for most small molecules.

Results:

The calibration curve showed good linearity for the range of 20.0 to 2000 ng/mL (refer to Figure 2). Peak symmetry (refer to Figure 3) was acceptable and the LLOQ standard was greater than 5 times signal to noise, therefore suitable for bioanalytical analysis. The method was not validated as per FDA guidelines.

Figure 2: Calibration Curve of Salmon Calcitonin

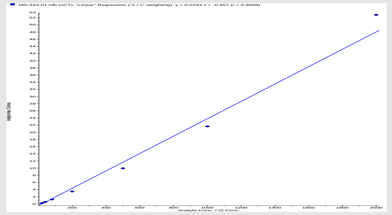
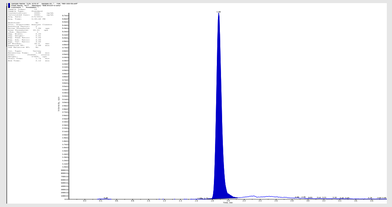


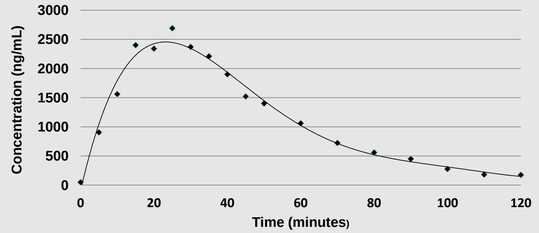
Figure 3: Injection of 20 ng per Spot Extract



The blood collecting process used for this in-vivo work was easily understood by the toxicokinetic technician. The mouse showed no undue stress to the numerous blood collections. The capillary tubes worked well for each blood collection and the spots generated were of similar size on the cards.

All 18 collected timepoints for the one mouse were analyzed on the same day, within 24 hours of sample collection. The results show a reliable TK profile is obtained (refer to Figure 4), that would be suitable to calculate TK parameters such as bioavailability and dose proportionality.

Figure 4: TK Profile from a Single Mouse



Discussion/Conclusion:

The results demonstrate it is possible to obtain a reliable TK profile from only one mouse instead of using multiple mice grouped together. Future work will need to demonstrate the variation obtained between different mice for proof of concept. Using the full volume spotted on the cards removes the risk of variance due to hematocrit levels, and may therefore make the approach of dry blood spots more viable in regards to any GLP studies being submitted to the FDA.

Acknowledgements:

Julie Tremblay, RLAT, ITR Laboratories Canada
Dominic Poulin, Study Director, ITR Laboratories Canada
John Chapdelaine, Head of Analytical Laboratory, ITR Laboratories Canada
Atsushi Nakai, Scientist, Analytical Laboratory, ITR Laboratories Canada
Babak Madadian, Analyst, Analytical Laboratory, ITR Laboratories Canada

References:

Rowland M., 2010. Use of Dried Blood Spots in Drug Development: Pharmacokinetic Considerations. AAPS Journal 12:290-293.
Keevil B., 2011. The analysis of dried blood spot samples using liquid chromatography tandem mass spectrometry. Clinical Biochemistry 44:110-118
Van den Broek I., Sparidans R.W., Schellens J.H.M, Beijnen J.H, 2008. Quantitative bioanalysis of peptides by liquid chromatography coupled to (tandem) mass spectrometry. Journal of Chromatography B 872:1-22