Quantitation of Diclofenac in Rat Skin Using LC-MS/MS Method after Collagenase Digestion and Bead Lysis
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**Purpose**

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) which is used to treat pain, inflammatory disorders and dysmenorrhea. Diclofenac is available as a generic drug in a number of formulations with different routes of administration. The occurrence of life threatening adverse events not only limits the usage of oral formulations but laid the path for the development of topical formulations. Permeation of drug through deeper tissue layers is essential to improve local drug delivery. The objective of the present study is to develop and validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of Diclofenac in rat skin to support pharmacokinetic studies after topical administration of equivalent daily doses.

**Methods**

As the skin is hard tissue, extraction of analytes from skin requires enzymatic pretreatment for its dissociation. Collagenase (Type II) with high levels of proteolytic activity is an effective and recommended methodology for tissue dissociation. The excised rat skin is incubated with 5 mg/mL collagenase prepared in 50 mM HEPES buffer at 37°C for 16 hrs and subsequently homogenized with addition of blend (0.9 to 2 mm) of stainless steel beads in a bullet blender (Next Advance, USA) for 5-10 minutes. Clean extracts are obtained by utilizing a liquid-liquid extraction of Diclofenac from 0.050 mL tissue homogenate sample with MTBE followed by evaporation and reconstitution. Diclofenac and Diclofenac-d4 (IS) were eluted from the reverse phase column (XBridge C18, 50 mm x 2.1 mm) by gradient elution technique at a flow rate of 0.6 mL/min. The mass spectrometry operating in negative ion mode through multiple reaction monitoring (MRM) was used for the detection of Diclofenac and IS.

**Results**

Diclofenac and IS are eluted at retention time of 2.50 min. The calibration standards prepared using rat skin tissue resulted in a linear calibration curve over the dynamic range of 0.25 – 250 µg/g with a correlation coefficient of 0.9969. The percentage recovery of Diclofenac from rat dermal tissue was found to be 62 ± 3.5% and the matrix factor was within acceptable limits.

**Conclusion**

Enzymatic digestion with collagenase in presence of calcium ions resulted in skin tissue dissociation. The method showed excellent precision with a % CV < 7.25 across all QC levels. This LC-MS/MS method has enabled the quantitation of Diclofenac skin concentrations after its topical application.