**Bioanalysis of Tirzepatide, a Dual GIP/GLP-1 Receptor Agonist, by LC-MS/MS and its Application to Pharmacokinetic Studies in Rats**

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**Background and aims.** Tirzepatide is the first dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor co-agonist, approved for the treatment of type 2 diabetes and obesity. Tirzepatide shows superior glycemic control and weight loss compared to conventional GLP-1 receptor agonists. This study aimed to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of tirzepatide and to characterize its pharmacokinetics in rats.

**Methods.** Tirzepatide was extracted from the rat plasma by simple protein precipitation with methanol. Chromatographic separation was achieved by gradient elution, with mobile phases composed of 0.1% formic acid in distilled water and acetonitrile. Mass spectrometry was conducted in positive electrospray ionization mode with optimized multiple reaction monitoring (MRM) transitions for tirzepatide quantification. The method was validated according to FDA bioanalytical guidelines. Sprague-Dawley rats were given tirzepatide by intravenous (IV) injection at 0.01, 0.05, and 0.5 mg/kg (n = 3-4) and by subcutaneous (SC) injection at 1 and 2 mg/kg (n = 4-6). Pharmacokinetic parameters were estimated using non-compartmental analysis.

**Results.** The developed LC–MS/MS method achieved a lower limit of quantification of 2 ng/mL with a linear calibration range of 2-2000 ng/mL. Intra- and inter-day accuracy ranged from 98.72% to 105.3%, with precision within 9.63%, satisfying regulatory guidelines for bioanalytical method validation. Following IV injection, plasma concentration of tirzepatide declined in a multi-exponential manner, with an average half-life of 9.42–10.27 h. Systemic exposure, as determined by the area under the curve (AUC), increased with dose. The subcutaneous bioavailability of tirzepatide was calculated to be 61.08-74.36% in rats.

**Conclusion/Discussion.** A robust and sensitive LC-MS/MS method was successfully developed and validated for the quantification of tirzepatide. This method enabled reliable characterization of tirzepatide pharmacokinetics in rats and provides a useful analytical platform to support future investigations, including formulation development and translational research.

**References:**

(1) Nauck, M. A. et al. (2022) Cardiovascular diabetology, 21:169