Application of a Gradient HPLC-UV Method for In Vitro Bioequivalence Assessment of Colesevelam Hydrochloride Tablet Formulations in Simulated Intestinal Fluid
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**Purpose**
The purpose of this study was to develop and validate a gradient HPLC-UV method for the quantitative determination of individual free (unbound) bile acids: Glycocholic Acid (GCA), Glycochenodeoxycholic Acid (GCDA) and Taurodeoxycholic Acid (TDCA) in aqueous solutions containing Colesevelam Hydrochloride and tablet excipients, as well as to identify and optimize the key process parameters to conduct both equilibrium and kinetic binding studies for in vitro bioequivalence evaluation against the marketed (reference) product.

**Methods**
A gradient HPLC method was developed and validated to quantify three bile acids in simulated intestinal fluid containing Colesevelam Hydrochloride formulations. Analysis was carried out using acetonitrile/10mM potassium phosphate buffer (pH 3.0) with starting composition of 55/45 %v/v as a mobile phase, in Waters C18 column (4.6 × 150 mm) with UV detection of 210 nm. The organic phase starts to ramp up at t=2min to 75% at t=10min to elute all three analytes, cleanly resolved from each other.

**Results**
Linearity of the method was found to be in the concentration range of 0.006-0.36mM, 0.011-0.66mM and 0.008-0.48mM for GCA, GCDA and TDCA respectively. The method was accurate with peak area %RSD (n=5) ≤ 2.0% and resolution ≥1.5, for each component. The binding experiments were performed using marketed Welchol 625mg Colesevelam tablets as directed in the FDA draft guidance. For the study parameters, the API-to-Incubation solution volume ratio was optimized to be 0.1mg/mL at both low (0.1mM) and high (15mM) total bile acid salt concentrations. The maximum binding was achieved at concentrations of 10mM and above. The 8 bile acid salt concentrations were defined, ranging from 0.5 to 25mM, which provide an adequate distribution of equilibrium binding results. The kinetic binding was determined to be 98.8% and 97% completed within 4 hours at the low (0.5mM) and high (25mM) concentrations, respectively.

**Conclusion**
A gradient HPLC method was developed suitable for use in bioequivalence studies for Colesevelam Hydrochloride tablet formulations. The study parameters for both equilibrium and kinetic binding experiments were optimized and finalized for in vitro bioequivalence assessment of generic products.