Method development for equilibrium and kinetic binding in vitro bioequivalence of Colesevelam Hydrochloride in simulated intestinal fluid (SIF)

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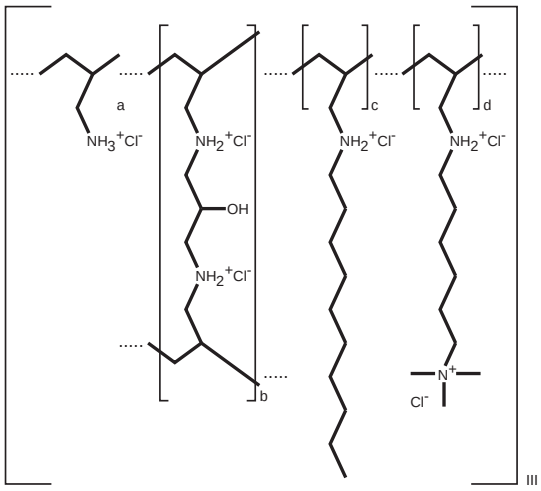
Introduction

Bioequivalence (BE) is defined as “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study”. If two products are said to be bioequivalent it means that they would be expected to be, for all intents and purposes, the same. Bioequivalence is usually established by in vivo studies, e.g. pharmacokinetic, pharmacodynamic, and clinical studies. Under certain circumstances, product bioequivalence can be documented using in vitro approaches. For example, those orally administered drugs intended for local action.



The objective of the study reported in this poster is to develop and validate an HPLC method for a bioequivalence study to quantitate free bile acid salts in aqueous solutions containing colesevelam hydrochloride and the excipients of Welchol® at various concentration levels.

Colesevelam hydrochloride is a high-capacity bile acid-binding molecule, which is a non-absorbed, polymeric, lipid-lowering and glucose-lowering agent, initially marketed as Welchol®.

Instrument and Settings

Agilent 1100 HPLC System with UV detection, Waters SymmetryShield™ RP18, 3.5 µm, 4.6 x 150 mm column.

Parameter	Value		
Flow Rate	1.0 mL/min		
Injection Volume	25 µL		
Column Temperature	30 ºC		
Autosampler Temperature	Ambient		
UV Wavelength	210 nm		
Attenuation	1000 mAU		
Gradient Profile A = 10mM KH_2PO_4, pH 3.0 B = Acetonitrile	**Time**	**%A**	**%B**
	0	55	45
	2	55	45
	10	25	75
	10.1	55	45
	12	55	45

Validation Experiment Design

The method was developed to determine the bile acid binding efficiency of a colesevelam hydrochloride formulation at different initial bile acid salt concentrations for an in vitro bioequivalence (BE) study. This method validation study intends to determine the remaining free bile acid salts (glycocholic acid, glycochenodeoxycholic acid, and taurodeoxycholic acid) after binding in the presence of an innovator product containing 625mg of colesevelam hydrochloride and excipients (Welchol®, Daiichi Sankyo, Inc.) at the initial bile acid salt concentration range of 0.1mM to 27.5mM. The assay solution concentration range to be validated is between 0.025mM to 4mM total bile acid salts, as this range is expected to encompass most experimental concentrations encountered in the BE study.
The validation consists of the following experiments: specificity, linearity of individual bile acid salts, LOQ confirmation, accuracy/recovery of samples, precision/repeatability, intermediate precision, standard and sample solution stability, and filter evaluation.

Validation Results

Specificity

Figure 1 presents an overlaid chromatogram of water, SIF, specificity matrix (ISE) and bile acid salts working standard solution (SA4). There are no interfering peaks observed in water, SIF or specificity solution at the retention time of each bile acid salt (GCA, GCDA and TDCA).

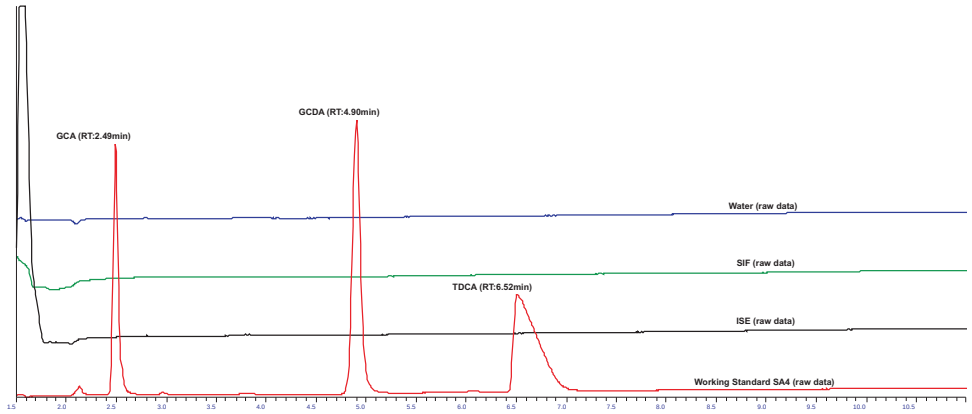
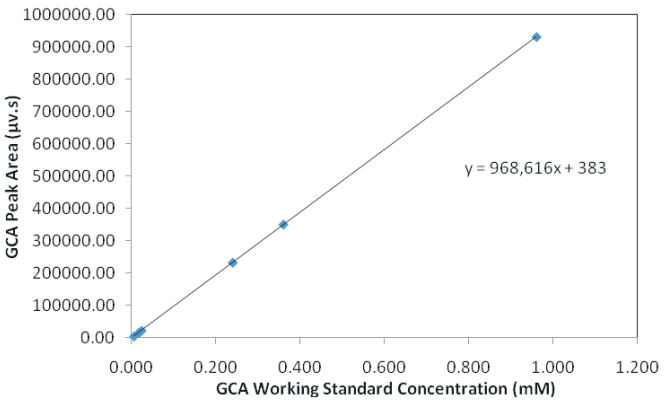
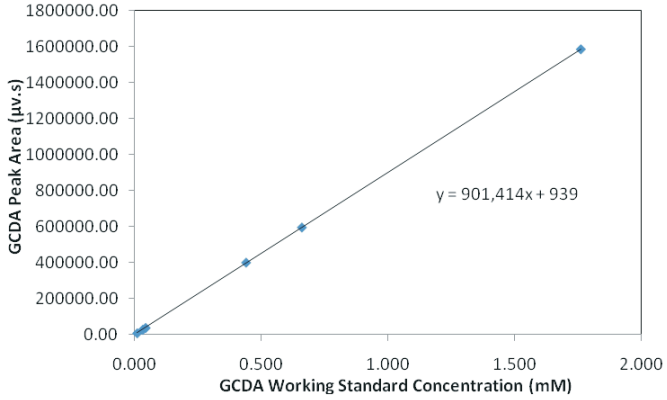


Figure 1. Overlaid Chromatogram of Specificity Analysis

Linearity/Range

Linearity was evaluated for reference standard solutions at the total bile acid salt concentration range between 0.025mM and 4mM. The linearity plots for all three individual bile acid salts are provided in Figure 2.





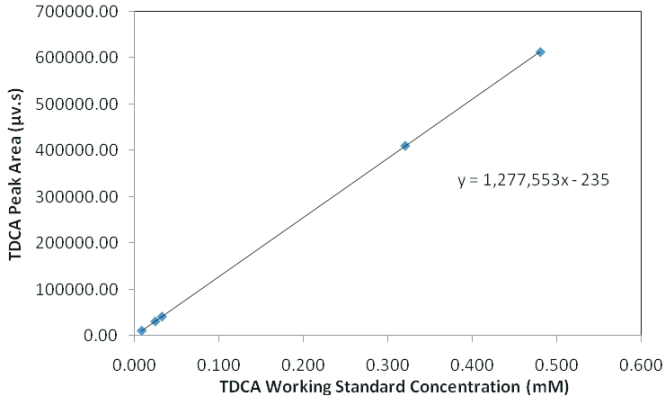


Figure 2. Linearity of Standard Solutions for GCA, GCDA and TDCA (from top to bottom)

Accuracy/Recovery
The accuracy/recovery of bile acid salts in aqueous solutions containing excipients of Welchol® formulation is demonstrated through % recovery at four different total bile acid salt concentrations (0.1, 1, 15 and 27.5mM as this range is expected to encompass most experimental concentrations to be encountered in the bioequivalence study). The low level of 0.1mM and 1mM samples were injected directly; the medium level of 15mM sample was diluted into assay concentration of 1.0mM and the high level of 27.5mM sample was diluted into assay concentration of 1.5mM. Three replicates were run for each concentration.

The accuracy/recovery data for each bile acid salt at different concentration levels are shown in Table 1.

Targeted Total Bile Salt Conc. (mM)	GCA Average %Recovery	GCA % RSD	GCDA Average %Recovery	GCDA % RSD	TDCA Average %Recovery	TDCA % RSD
0.1	104.6	1.2	98.4	2.3	96.3	2.6
1	102.0	0.1	101.0	0.1	98.2	0.1
15	100.0	0.2	99.5	0.1	99.2	0.1
27.5	100.0	1.8	99.9	1.7	99.4	1.7

Table 1. Accuracy/Recovery and Method Precision Results

BE Study Development

Following the testing outlined in the FDA Draft Guidance for colesevelam hydrochloride, both in vitro equilibrium binding (with and without acid pre-treatment) and in vitro kinetic binding studies should be conducted.

Simulated Intestinal Fluid (SIF), a 0.05M potassium phosphate buffer solution prepared in water and adjusted to pH 6.8 with 0.2N NaOH, serves as the assay buffer for equilibrium and kinetic binding experiments. The bile acid salts are present in a molar proportion of 3:3:1 (GCA:GCDA:TDCA) in SIF (ratio from FDA draft Guidance for Cholestyramine). 12 replicates each of test and reference product are evaluated at each condition detailed below.

For in vitro equilibrium binding, a fixed drug product equivalent of API is combined with incubation solution at various bile acid salt concentrations. Solutions are incubated at 37°C, shaking at 150 rpm. Aliquots are filtered prior to further dilution or vialing for HPLC analysis (conducted for all experiments).

Per the FDA draft guidance document, the impact of acid pre-treating colesevelam hydrochloride should also be evaluated in equilibrium binding. For acid pre-treatment, the same fixed drug product equivalent of API is incubated in HCl at 37°C. Following incubation, preparations are vacuum-filtered and rinsed with sufficient SIF until the filtrate reaches pH 6.8. Samples are transferred to incubation flasks containing incubation solution at various bile acid salt concentrations and subjected to equilibrium binding evaluation.

For the kinetic binding study, samples are prepared using API in incubation solution at the low and high equilibrium binding bile acid salt concentrations. The degree of binding is determined at multiple time intervals.

Conclusion

The validated method met all acceptance criteria and is suitable for use in BE studies.