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Letter to the Editor

In vitro Bioequivalence Studies for Phosphate Binding Drugs: Analytical Method Validation Approaches

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Bioequivalence (BE) testing plays an important role in the development and approval of generic drugs and is usually assessed by measuring the rate and extent to which the drug product is absorbed into the blood stream [1]. The current BE approach is not applicable for locally acting gastro intestinal phosphate-binding drugs such as Sevelamer carbonate and Lanthanum carbonate since they are not intended to be absorbed into the systemic circulation. The drug concentration needs to be estimated at the local GI tract site. These drugs dissociate in the acid environment of the upper GI tract to release ionic drug species that bind to dietary phosphate to form an insoluble complex that is eliminated via faeces. Therefore, BE studies of phosphate binding drugs pose a major challenge for the pharmaceuticals industry and regulatory authorities [2].

The FDA has developed a set of guidelines for *In vitro* BE studies of phosphate binding drugs that include *In vitro* equilibrium and kinetic phosphate binding studies to compare the extent and rate of binding affinity between Test and Reference formulations. Prior to conducting the equilibrium study, the maximum (or saturation) phosphate binding concentration need to be established at recommended pH. The equilibrium binding study is subsequently performed by incubating Test and Reference formulations for a constant period of time with at least eight phosphate concentrations. The kinetic binding study is carried out at high and low phosphate concentrations over varying time periods at 37°C. It is also recommended to conduct equilibrium and kinetic studies with inclusion of an acid pretreatment condition (pH 1.2). Langmuir binding constants k1 and k2 are established from the equilibrium binding study, where k1is derived by comparing the Test and Reference samples and an appropriate confidence interval is calculated for k2 [3-5].

Well developed and validated analytical methods are the foundation of *In vitro* BE studies aimed at predicting the performance of phosphate-binding drugs in the GI tract. Ion chromatography, high performance capillary electrophoresis and inductive coupled plasma atomic emission spectroscopy (ICP-AES) are commonly employed to estimate the free phosphate concentration for *In vitro* BE studies [6-8]. However, there are no specific recommended analytical validation protocols to conduct the *In vitro* BE studies. Thus, a real and urgent need exists to establish a fully validated analytical method and standard test protocols for *In vitro* BE studies to enable reproducible comparison of Test formulations with a high level of confidence. At present, analytical method validation parameters and acceptance criteria are adopting from *in vivo* bio-analytical methodology.

Proposed validation parameters for *In vitro* BE studies include Specificity, Placebo binding, Carryover checks, Linearity, Accuracy and Precision, Recovery, Stability (Bench-Top Stability, Processed Sample Stability and Stock Solution Stability), Ruggedness, Robustness and Filter validation. The specificity of the method needs to be established for placebo and sample blanks of both Test and Reference products at a specified pH condition. Blank (or placebo) binding studies will enable phosphate binding to be evaluated for the inactive excipients in both Test and Reference products. Construction of calibration curves and measurements of the lower limit of quantification (LLOQ), accuracy and precision need to be carried out using placebo and the methodology can only be accepted when there is no effect of the excipients on phosphate binding. Stability studies need to be conducted at low, medium and high phosphate concentrations corresponding to values selected for the equilibrium study. Initial day, unbound phosphate data may be considered as the nominal concentration of the samples, whereas calibration standard stability needs to be ascertained using actual phosphate concentrations. It is not possible to measure recovery from *In vitro* BE studies of equilibrium phosphate binding since the method determines unbound phosphate levels. Recovery can only be calculated from Accuracy and Precision measurements. Robustness is determined for the particular method employed (e.g., by characterizing exactly the influence of mobile phase pH, composition and temperature for ion chromatography or RF power and sample flow rate for ICP-AES). Ruggedness is established by using a different analyst to perform the assay. Investigations of filter performance are essential to confirm the absence of phosphate adsorption to the filters to ensure correct assignment of LLOQ, accuracy and precision [9, 10].

A major challenge is to establish the acceptance criteria for all aforementioned validation parameters. Five replicates can be considered adequate for each validation parameter. These criteria can also vary simply because of the analytical methodology employed; for example analysis of phosphate concentration by ion chromatography is expected to be more reproducible compared with ICP-AES. Estimation of the drug concentration *in vivo* is much more demanding because of the complex biochemical and physiological environment compared with the simple model used for *In vitro* BE studies [11]. These difficulties have prompted regulatory authorities to recommend that the obtained validation results should be within 15% of the nominal value, except at the LLOQ, where it should not deviate by more than 20%. As usual, the prime question that need to be addressed is how relevant are *in vitro* BE studies to *in vivo* analytical

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methods and the prediction of *in vivo* drug performance. The answer will only be found by ongoing rigorous and critical appraisals of current *In vitro* BE methods in comparison with *in vivo* behavior.

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