the two periods to allow the subjects to completely eliminate the drug absorbed from the first dose before administration of the second dose. Also, for long half-life drugs, a single-dose parallel design may be used.[40] For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours may be used in place of AUC_{0-t} or AUC_{∞}.[26]

Number of Subjects; Single-Dose versus Steady-State Bioequivalence Studies

The FDA recommends that investigators enroll a minimum of 12 subjects.[25] Most bioequivalence studies submitted in support of ANDAs enroll from 24 to 36 subjects. The FDA asks investigators to conduct single-dose bioequivalence studies because it has been shown that these are more sensitive to detecting differences in formulation performance than multiple-dose studies.[26,41–45]

Appropriate Drug Product Strength for Bioequivalence Studies

Most bioequivalence studies are conducted on the highest strength of a drug product line, unless it is necessary to use a lower strength for safety reasons. Use of the highest strength is particularly critical for drugs that display nonlinear kinetics because of nonlinear (usually capacity-limited) elimination or presystemic metabolism, with the result that plasma concentrations increase more than proportionally with an increase in dose.[46] For such drugs, small differences in the rate or extent of absorption can potentially have substantial effects on the AUC.[47] Thus, using the highest strength in bioequivalence studies or, in some cases, the highest starting dose—so that drug pharmacokinetics are potentially in the "nonlinear range"—ensures that a generic formulation will not pass bioequivalence acceptance criteria unless it is formulated to provide nearly the same rate and extent of exposure as the corresponding reference product. For drugs for which rate and/or extent of absorption increases less than proportionally with an increase in dose,[48] the bioequivalence study will be most discriminating if conducted at the lowest strength or, if only one strength is marketed, at the lowest recommended dose.

Fed Bioequivalence Studies

Because food can influence the bioavailability of orally administered drugs, the FDA recommends that applicants conduct bioequivalence studies under fed conditions in most cases. The FDA's Guidance for Industry, *Food-Effect Studies and Fed Bioequivalence Studies* ("Food Guidance"), contains recommendations about designing fed bioequivalence studies.[49] Fed bioequivalence studies are generally conducted using meal conditions expected to provide the greatest effects on formulation performance and gastrointestinal physiology such that systemic drug bioavailability may be maximally affected. Typically, the drug is administered to subjects within 30 minutes of consuming a high-fat, high-calorie meal. The FDA recommends that these studies use a randomized, balanced, single-dose, two-treatment (fed test vs. fed reference), two-period, two-sequence crossover design. The acceptance criteria for fed bioequivalence studies is the same as for fasting bioequivalence studies—the