appropriate reference material conducted in normal adults.

(2) The test product and the reference material should be administered to subjects in the fasting state, unless some other approach is more appropriate for valid scientific reasons.

(b) *Study design.* (1) A single-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period.

(2) Unless some other approach is appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured in the blood or urine; or

(ii) At least three times the half-life of decay of the acute pharmacological effect.

(c) *Collection of blood samples.* (1) When comparison of the test product and the reference material is to be based on blood concentration time curves, unless some other approach is more appropriate for valid scientific reasons, blood samples should be taken with sufficient frequency to permit an estimate of both:

(i) The peak concentration in the blood of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured; and

(ii) The total area under the curve for a time period at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(2) In a study comparing oral dosage forms, the sampling times should be identical.

(3) In a study comparing an intravenous dosage form and an oral dosage form, the sampling times should be those needed to describe both:

(i) The distribution and elimination phase of the intravenous dosage form; and

(ii) The absorption and elimination phase of the oral dosage form.

(4) In a study comparing drug delivery systems other than oral or intravenous dosage forms with an appropriate reference standard, the sampling times should be based on valid scientific reasons.

(d) *Collection of urine samples.* When comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves, unless some other approach is more appropriate for valid scientific reasons, samples of the urine should be collected with sufficient frequency to permit an estimate of the rate and extent of urinary excretion of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(e) *Measurement of an acute pharmacological effect.* (1) When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to permit a reasonable estimate of the total area under the curve for a time period at least three times the half-life of decay of the pharmacological effect, unless some other approach is more appropriate for valid scientific reasons.

(2) The use of an acute pharmacological effect to determine bioavailability may further require demonstration of dose-related response. In such a case, bioavailability may be determined by comparison of the dose-response curves as well as the total area under the acute pharmacological effect-time curves for any given dose.

[42 FR 1648, Jan. 7, 1977, as amended at 67 FR 77674, Dec. 19, 2002]

§ 320.27 Guidelines on the design of a multiple-dose in vivo bioavailability study.

(a) *Basic principles.* (1) In selected circumstances it may be necessary for the test product and the reference material to be compared after repeated administration to determine steady-state levels of the active drug ingredient or therapeutic moiety in the body.

(2) The test product and the reference material should be administered to subjects in the fasting or nonfasting state, depending upon the conditions reflected in the proposed labeling of the test product.

(3) A multiple-dose study may be required to determine the bioavailability of a drug product in the following circumstances:

(i) There is a difference in the rate of absorption but not in the extent of absorption.

(ii) There is excessive variability in bioavailability from subject to subject.

(iii) The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood resulting from a single dose is too low for accurate determination by the analytical method.

(iv) The drug product is an extended release dosage form.

(b) *Study design.* (1) A multiple-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period if steady-state conditions are not achieved.

(2) A multiple-dose study is not required to be of crossover design if the study is to establish dose proportionality under a multiple-dose regimen or to establish the pharmacokinetic profile of a new drug product, a new drug delivery system, or an extended release dosage form.

(3) If a drug elimination period is required, unless some other approach is more appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least five times the half-life of the active drug ingredient or therapeutic moiety, or its active metabolite(s), measured in the blood or urine; or

(ii) At least five times the half-life of decay of the acute pharmacological effect.

(c) *Achievement of steady-state conditions.* Whenever a multiple-dose study is conducted, unless some other approach is more appropriate for valid scientific reasons, sufficient doses of the test product and reference material should be administered in accordance with the labeling to achieve steady-state conditions.

(d) *Collection of blood or urine samples.* (1) Whenever comparison of the test product and the reference material is to be based on blood concentration-time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.

(2) Whenever comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.

(3) A more complete characterization of the blood concentration or urinary excretion rate during the absorption and elimination phases of a single dose administered at steady-state is encouraged to permit estimation of the total area under concentration-time curves or cumulative urinary excretion-time curves and to obtain pharmacokinetic information, e.g., half-life or blood clearance, that is essential in preparing adequate labeling for the drug product.

(e) *Steady-state parameters.* (1) In certain instances, e.g., in a study involving a new drug entity, blood clearances at steady-state obtained in a multiple-dose study should be compared to blood clearances obtained in a single-dose study to support adequate dosage recommendations.

(2) In a linear system, the area under the blood concentration-time curve during a dosing interval in a multiple-dose steady-state study is directly proportional to the fraction of the dose absorbed and is equal to the corresponding ''zero to infinity'' area under the curve for a single-dose study. Therefore, when steady-state conditions are achieved, a comparison of blood concentrations during a dosing interval may be used to define the fraction of the active drug ingredient or therapeutic moiety absorbed.

(3) Other methods based on valid scientific reasons should be used to determine the bioavailability of a drug product having dose-dependent kinetics (non-linear system).

(f) *Measurement of an acute pharmacological effect.* When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to demonstrate a maximum effect and a lack of significant difference between the

test product and the reference material.

[42 FR 1648, Jan. 7, 1977, as amended at 67 FR 77674, Dec. 19, 2002]

§ 320.28 Correlation of bioavailability with an acute pharmacological effect or clinical evidence.

Correlation of in vivo bioavailability data with an acute pharmacological effect or clinical evidence of safety and effectiveness may be required if needed to establish the clinical significance of a special claim, e.g., in the case of an extended release preparation.

[42 FR 1648, Jan. 7, 1977, as amended at 67 FR 77674, Dec. 19, 2002]

§ 320.29 Analytical methods for an in vivo bioavailability or bioequivalence study.

(a) The analytical method used in an in vivo bioavailability or bioequivalence study to measure the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products, or the method used to measure an acute pharmacological effect shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate precision, the actual concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), achieved in the body.

(b) When the analytical method is not sensitive enough to measure accurately the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products produced by a single dose of the test product, two or more single doses may be given together to produce higher concentration if the requirements of § 320.31 are met.

[42 FR 1648, Jan. 7, 1977, as amended at 67 FR 77674, Dec. 19, 2002]

§ 320.30 Inquiries regarding bioavailability and bioequivalence requirements and review of protocols by the Food and Drug Administration.

(a) The Commissioner of Food and Drugs strongly recommends that, to avoid the conduct of an improper study and unnecessary human research, any person planning to conduct a bioavailability or bioequivalence study submit the proposed protocol for the study to FDA for review prior to the initiation of the study.

(b) FDA may review a proposed protocol for a bioavailability or bioequivalence study and will offer advice with respect to whether the following conditions are met:

(1) The design of the proposed bioavailability or bioequivalence study is appropriate.

(2) The reference material to be used in the bioavailability or bioequivalence study is appropriate.

(3) The proposed chemical and statistical analytical methods are adequate.

(c)(1) General inquiries relating to in vivo bioavailability requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Office of Clinical Pharmacology, 10903 New Hampshire Ave., Silver Spring, MD 20993–0002.

(2) General inquiries relating to bioequivalence requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Bioequivalence (HFD–650), 7500 Standish Pl., Rockville, MD 20855–2773.

[57 FR 18000, Apr. 28, 1992, as amended at 67 FR 77674, Dec. 19, 2002; 74 FR 13114, Mar. 26, 2009]

§ 320.31 Applicability of requirements regarding an "Investigational New Drug Application."

(a) Any person planning to conduct an in vivo bioavailability or bioequivalence study in humans shall submit an ''Investigational New Drug Application'' (IND) if:

(1) The test product contains a new chemical entity as defined in § 314.108(a) of this chapter; or

(2) The study involves a radioactively labeled drug product; or

(3) The study involves a cytotoxic drug product.

(b) Any person planning to conduct a bioavailability or bioequivalence study in humans using a drug product that contains an already approved, non-new chemical entity shall submit an IND if the study is one of the following: