

Sponsors/applicants may select compounds from the list of drugs and/or chemicals provided in Attachment A, or they may select other drugs for which there is information available on mechanism of absorption and reliable estimates of the extent of drug absorption in humans.

For a given test method with set conditions, selection of a high permeability internal standard with permeability in close proximity to the low/high permeability class boundary may be used to facilitate classification of a test drug substance. For instance, a test drug substance may be determined to be highly permeable when its permeability value is equal to or greater than that of the selected internal standard with high permeability.

After demonstrating suitability of a method and maintaining the same study protocol, it is not necessary to retest all selected model drugs for subsequent studies intended to classify a drug substance. Instead, a low and a high permeability model drug should be used as internal standards (i.e., included in the perfusion fluid or donor fluid along with the test drug substance). These two internal standards are in addition to the fluid volume marker (or a zero-permeability compound such as PEG 4000) that is included in certain types of perfusion techniques (e.g., closed loop techniques). The choice of internal standards should be based on compatibility with the test drug substance (i.e., they should not exhibit any significant physical, chemical, or permeation interactions). When it is not feasible to follow this protocol, the permeability of internal standards should be determined in the same subjects, animals, tissues, or monolayers, following (or, if appropriate, in parallel to) evaluation of the test drug substance. The permeability values of the two internal standards should not differ substantially between experiments conducted to demonstrate the assay's method suitability and those for the test drug. For example, the laboratory may set acceptance criteria for the permeability values of its high, low, and zero permeability standard compounds.

At the end of an *in vitro* test, the amount of drug in the tissue or cell monolayer, apical and basolateral chambers should be determined to assist in calculation of mass balance. If recovery from the apical and basolateral chambers is >80%, there is no need to measure drug in the tissue or cell monolayers.

When intestinal permeability methods are used to demonstrate high permeability, additional data to document the drug's stability in the GI tract is required. Please see method details in section III.B.3.

Instability in the Gastrointestinal Tract

Determining the extent of absorption in humans based on mass balance studies using total radioactivity in urine does not take into consideration the extent of degradation of a drug in the GI fluid prior to intestinal membrane permeation. In addition, some methods for determining permeability could be based on loss or clearance of a drug from fluids perfused into the human and/or animal GI tract either *in vivo* or *in situ*. Documenting the fact that drug loss from the GI tract arises from intestinal membrane permeation, rather than a degradation process, will help establish permeability. Stability in the GI tract may be documented using simulated gastric and intestinal fluids. Obtaining GI fluids from human subjects requires intubation and may be difficult. Stability in the GI tract may therefore be documented using simulated gastric and intestinal fluids such as Gastric and Intestinal Fluids USP or, with suitable justification, other biorelevant media.