

Comparator Development & Validation

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Introduction

A comparator product is a drug product in development or a finished pharmaceutical (active or placebo) that is compared to another product of the same therapeutic class for the purposes of studying bioequivalency, efficacy, and safety. Analytical comparative testing is important as a reference to clinical testing as well as to demonstrate that the product of interest is equivalent or superior to a marketed brand of the same drug class. Companies often seek to outsource the analytical support involved in the comparative testing of their products. This analytical support includes, but is not limited to development of methods for assay, dissolution, and moisture testing, validation of these methods, and release and stability testing of the comparator.

Overview

QTI has fifteen years of experience working with comparative agents. Due to this experience, an array of services is available. QTI has resources and capabilities for the manufacture of blinded products by over encapsulation, or de-inking, expertise in development and validation of methods for assay, dissolution, and moisture testing, and facilities for release and stability testing.

Manufacture of Dosage Units

Innovator commercial products can be over encapsulated either with or without backfill. A wide range of capsules shell sizes are available to accommodate drug products ranging from 1 mg to 875 mg of active product. The backfills used in comparator studies consist of inert powders (typically sugars) such as anhydrous lactose or lactose monohydrate. Microcrystalline cellulose is also used in cases where the clinical test group has aversions to lactose. All dosage units are prepared by hand. The first 20 capsules are prepared by placing the commercial tablet into the body of the capsule. The void space around the tablet is then completely filled with the appropriate backfill if necessary. The capsule is then capped and locked and wiped clean with a laboratory wipe. An average weight is obtained for the first 20 units. Each additional unit that is manufactured is weighed individually and must be within $\pm 5\%$ of the average weight determined from the first 20. This ensures consistency and uniformity between the dosage units. Any dosage unit that falls outside of the weight range is excluded from testing. During this process each commercial tablet and capsule shell is inspected for defects. If a tablet is found to be cracked or chipped it is discarded. Any capsules shells with cracks or dents are not used. If necessary, dissolution blanks can also be prepared in this same manner without the commercial tablet.

Assay Method Development

Assay method development is important for any product that will be placed on stability. These methods allow for tracking of impurities and degradation products and can also be used to explain changes in a product's release profile. QTI SOP-000495 governs development of analytical methods for determination of assay and impurities in a drug product. This document was written based on guidelines set forth by the USP and FDA. For the purposes of comparator testing, the innovator's product is used as a reference material for method development.

Diluent Evaluation

The first goal in the development process is to determine what the compound of interest is soluble in. In assay methods the dissolving or extraction solvent is typically some combination of water and organic solvent such as methanol, acetonitrile or ethanol. An initial solubility experiment would include preparation of several standards using varied ratios of these solvents. Extraction of a compound may also employ techniques such as sonication, shaking, and clarification by centrifugation.

Standard and Sample Extraction

When compendial reference materials are not available for a particular product, standard solutions can be prepared from a composite of commercial tablets. In such cases an optimal extraction technique must be chosen. Once an appropriate diluent is chosen, the tablet composite is extracted using a combination of sonication to break up and dissolve the active component, mechanical shaking to disperse and homogenize the suspension and clarification by centrifugation. During this experiment several standards are prepared using varied sonication times to determine the amount needed to obtain complete extraction. Dilutions can be made from the clarified stock in order to obtain the desired concentration. Following development of HPLC-UV parameters, all solutions are analyzed to determine the most effective procedure for preparing the standard solution.

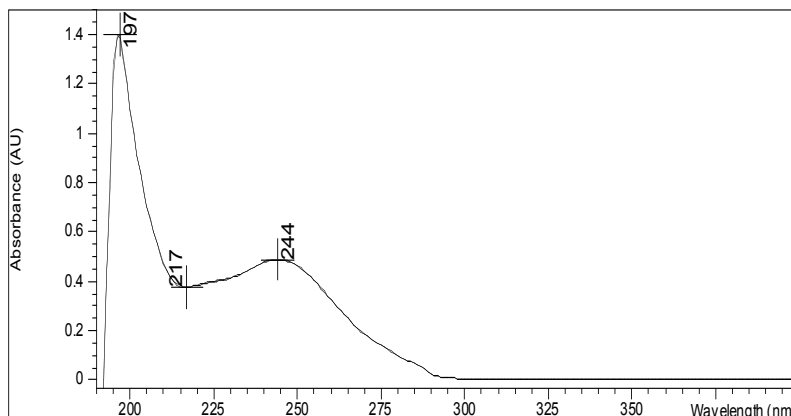
A preparation technique must also be developed for the blinded samples. Over encapsulated samples are often prepared and extracted using a diluent and procedure similar to preparing working standards from reference material. No less than 5 capsules should be extracted in an appropriate volume of diluent. Varied amounts of sonication and shaking are employed to thoroughly break up the dosage units and extract the active compound. Solutions are clarified by centrifugation until a suitable filter is chosen during the validation process. Dilutions of the stock solutions can be made to obtain the desired working concentration.

Determination of HPLC Parameters

Detection Wavelength

A working standard solution prepared at the nominal sample concentration is analyzed by UV spectrophotometry to determine an appropriate wavelength for analysis. The solution is scanned and a UV spectrum is taken from 190 nm to 400 nm. An area of maximum or minimum absorbance is chosen that will provide adequate and consistent response for the compound of interest. If the compound of interest does not have a chromophore and therefore does not absorb UV light, other methods of detection such as fluorescence, evaporative light scattering, or mass spectrometry should be evaluated.

Figure 1. Example UV Spectrum



HPLC Column

In reversed phase HPLC, the column or stationary phase consists of hydrophobic material such as long alkyl chains. For most pharmaceuticals, L1 packing (C8 or C18) provides adequate retention of the molecule. Special bonded phases are used to retain compounds that have special functional groups such as amines, phenyl rings, or cyano groups. Columns that employ silica/siloxo-ethane technology such as the Waters X-Bridge or the Phenomenex Gemini are useful for development purposes because the hybrid packing material is stable over a broad range of pH (1 to 12).

Mobile Phase

In reverse phase HPLC, the composition of the mobile phase is dependent on the behavior of the drug molecule in solution. For assay methods, the mobile phase usually consists of two components, an aqueous portion and an organic portion. For compounds that form ions in solution, the aqueous portion of the mobile phase should be pH adjusted to at least ± 1 unit beyond the pKa of the compound. This ensures that the compound will either be completely ionized or completely neutral as it encounters the column. Molecules that are basic in solution will be more retained when the mobile phase pH is higher and vice versa. Acidic molecules will be more retained when the mobile phase is lower and vice versa. The organic portion of the mobile phase is typically acetonitrile, methanol, or a combination of the two. Once the mobile phase components are chosen, a scouting gradient is performed where the mobile phase is mixed by the HPLC pump and the percent of organic in the mixture is slowly increased from 5% to 95%. This gradient along with the recommended starting conditions will provide an initial retention and resolution for the active.

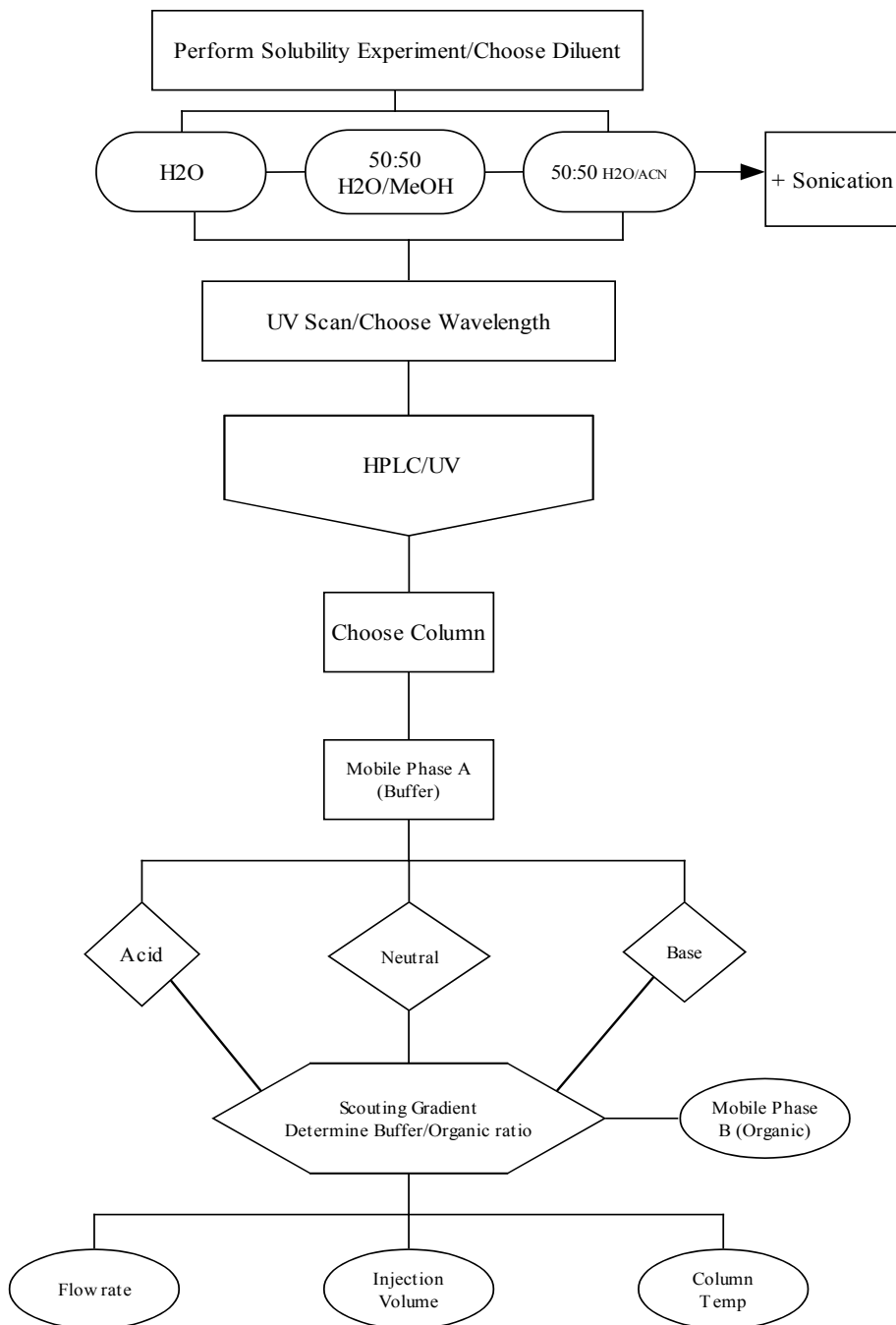
Table 1. Recommended Parameters for Initial HPLC Development

Parameter	Condition
Column	Waters X-Bridge, C18, 100 mm X 3.0 mm, 3.5 μ m particle size
Mobile Phase	Mobile Phase A = 0.1% Trifluoroacetic acid Mobile Phase B = Acetonitrile/Methanol
Column Temp	40 $^{\circ}$ C
Flow Rate	1.0 mL/min
Injection Volume	10 μ L
Run Time	30 minutes

Optimization of Instrument Parameters

Following the performance of the initial gradient, the HPLC parameters can be adjusted to provide adequate peak response, retention, peak shape, and resolution from adjacent peaks. Injection volume may be increased to increase peak response or decreased to bring a peak on scale. The gradient slope can be adjusted and/or steps can be added in order to increase resolution from adjacent interferences. The column temperature may be increased or decreased to improve peak shape and to improve resolution in some instances. The system flow rate may be increased to reduce overall run time if resolution and retention are adequate.

Figure 2. Decision Tree for Assay Method Development



Pre-validation Experiments

Once the instrument parameters have been developed, some minimal pre-validation experiments are performed to determine if the method will need further optimization prior to validation.

- Specificity is performed to determine if the method is specific for the compound of interest and if there is any interference from the diluent or blinding components.
 - This experiment would also include limited forced degradation of the compound using dilute acid, base, and peroxide. Degradation products are purposely created and analyzed to determine if the method provides adequate resolution from potential degradation products.
- A preliminary linearity experiment is performed to ensure that the method will be linear over a specific range of concentrations.
- The limit of detection and limit of quantitation are determined for the method.
 - ICH guidelines defines limit of detection as any peak greater than or equal to three times the baseline noise. Limit of quantitation is defined as any peak greater than or equal to ten times the baseline noise.
 - The percentage of the nominal sample concentration is calculated for both the limit of detection and limit of quantitation.

Dissolution Method Development

QTI SOP-000496 governs development of analytical methods for determination of the dissolution rate of a drug product by reversed phase HPLC/UV. This document was written based on guidelines set forth by the USP and FDA. For the purposes of comparator testing, the innovator's product is used as a reference material for method development.

Determination of HPLC Parameters

For the purposes of testing dissolution samples, HPLC instrument parameters will be based on those determined during assay method development. The mobile phase gradient would be eliminated and the two mobile phase components would be hand mixed at the approximate ratio of aqueous to organic where the compound of interest elutes. A higher flow rate and/or shorter HPLC column may be chosen to shorten the overall chromatographic run time. It is very important that the method provides for ample sample throughput and for this reason the HPLC method should have a run time no longer than 10 minutes.

Choosing a Dissolution Medium and Conditions

For comparator development, three dissolution media are tested to cover the biologically relevant spectrum. These media are 0.1N HCl (simulated gastric fluid without enzymes), pH 4.5 acetate buffer, which is tested as an intermediate pH, and pH 6.8 phosphate buffer (simulated intestinal fluid without enzymes). An experiment should be conducted to determine the solubility of the compound in each of the three experimental media. During this experiment sonication can be used (up to 30 minutes) to aid in dissolving the active and organic solvent can be added up to 10% of the working solution. If the compound proves to be insoluble in one or more of the media even with sonication and the addition of organic, this would exclude those media from additional testing. Additional media containing surfactants such as sodium dodecyl sulfate or polysorbate 80 can be used to increase solubility in place of any of the three experimental media. Surfactants are typically added over a range of 0.1% up to 2.0% w/v to determine optimal solubility.

An important factor in the development of any dissolution method is the establishment of sink conditions. Sink conditions are defined as three times the volume needed to produce a saturated solution of drug. In situations where reference material is available for use, sink can be determined by adding the drug material to a known volume of dissolution medium until precipitation occurs. This solution is then analyzed against a known standard by UV or HPLC/UV to determine the concentration of the saturated solution. The resulting concentration is divided by three and compared to the nominal concentration. If the concentration of the saturated solution is greater than or equal to three times the nominal sample concentration then sink conditions are present.

If reference material is not available, linearity standards should be prepared from composite up to and including three times the nominal sample concentration. The linearity solutions are then analyzed by UV or HPLC/UV and a linear regression is plotted. If the plot is linear, then sink conditions are present. If the line plateaus at any point then sink has not been established. If sink conditions are not present, the volume of dissolution medium can be increased or other media can be evaluated.

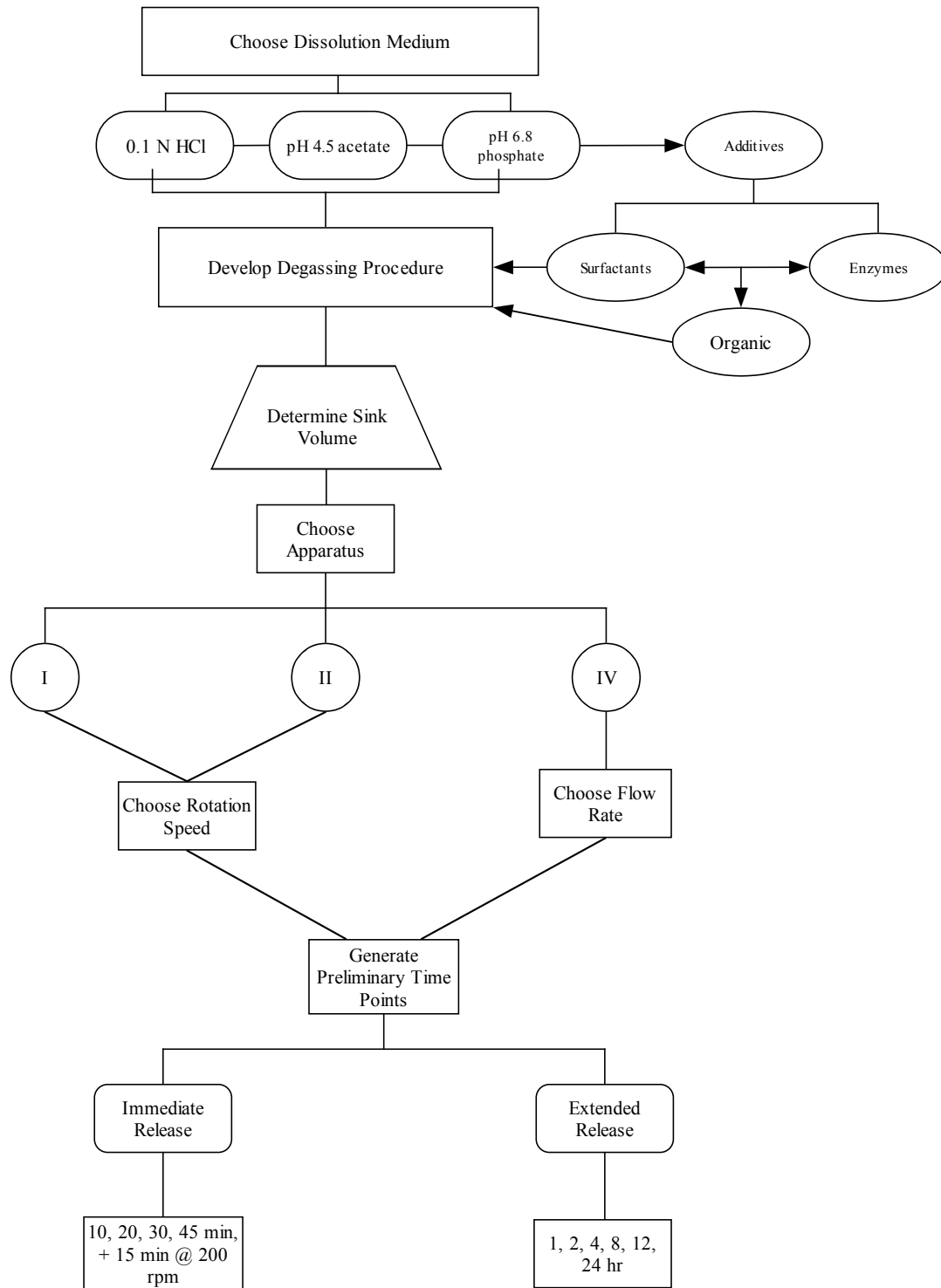
Dissolution media can be degassed using a variety of procedures. One easy and effective technique is using helium sparge with stirring for 0.5 minutes per liter of solution. Other techniques include stirring under vacuum, and filtering under vacuum at 37°C. Degassing is discouraged for media containing surfactants due to the amount of foaming that is inherently produced.

Table 2. Recommended Conditions for Dissolution Development

Parameter	Condition
Dissolution Medium	0.1N HCl, pH 4.5 acetate, pH 6.8 phosphate
Volume	900 mL-after verifying sink
Apparatus	I (rotating baskets)
Rotation	100 rpm
Temperature	37 °C
Time pulls	15, 30, 45, 60, +15 @ 200 rpm

For most immediate release products, using Apparatus I (rotating baskets) at 100 rpm provides acceptable percent dissolved results by a Q time of 30 minutes. If during the performance of the dissolution, it is observed that the baskets become clogged with capsule material or excipients, the basket mesh size can be changed or the dissolution can be performed using Apparatus II (rotating paddles) using capsule weighting devices. There are several other viable apparatuses for comparative testing such as Apparatus III (reciprocating cylinder) or Apparatus IV (flow through cell). The dissolution is maintained at 37°C to mimic internal body temperature. For immediate release dosage forms an initial release profile would be generated for the blinded sample and corresponding dissolution blank (n = 3). Samples are pulled at 15, 30, 45, and 60 minutes at the method rotation and a recovery time point of + 15 minutes at 200 rpm. The recovery time point is inserted as a reference for complete dissolution of the drug product. Profiles generated for delayed or controlled release products would contain at least three time points covering the entire duration of the drug release.

Figure 3. Decision Tree for Dissolution Method Development



Method Validation

Method validation is governed by QTI SOP-000036 and is based upon ICH and USP guidelines.

Assay Method Validation

During validation of the assay method parameters such as filter qualification, injection precision, method precision, linearity, accuracy, and solution stability are evaluated. An expanded forced degradation should also be included that stresses the drug product with heat and light. If necessary, chromatographic robustness can also be evaluated by making small changes to the instrument parameters and determining the impact on system suitability and sample assay.

Dissolution Method Validation

During validation of the dissolution method parameters such as specificity, filter qualification, system precision, linearity, accuracy, and solution stability are evaluated. Method precision is also evaluated by testing the blinded product versus the commercial tablets ($n = 6$). The FDA guideline for immediate release products states that the dissolution profiles are considered similar if both dosage forms exhibit $\geq 85\%$ dissolved at 30 minutes. If necessary, dissolution robustness can also be evaluated by making small changes to the instrument parameters and determining the impact on the dissolution profile and drug release.

Moisture Determination by Karl Fischer Titration

Products that absorb moisture from the environment will need to be monitored during release and stability testing. For this purpose, a method must be developed and validated for the determination of moisture within the drug product. Karl Fischer titration is an effective tool in performing this function.

Direct addition (volumetric) titration is used for products that typically contain 1 to 100 mg per sample. During volumetric titration solid or liquid samples are added directly to the titration cell, which contains a dissolving solvent such as methanol. The sample is then stirred and titrated using a composite solution of imidazole, sulfur dioxide, and hydroiodic acid. In developing and validating a method for volumetric titration of a sample composite, method parameters such as instrument stir time, sample size linearity, and reproducibility ($n = 5$) are evaluated.

The Karl Fischer coulometric method is used to determine the moisture in samples containing 10 μg to 10 mg of water per sample. In the coulometric method the sample is first dissolved or extracted in a chosen diluent. The supernatant of this solution is then injected into the closed titration cell. Iodine plus two electrons are generated in the titration cell by anodic oxidation of iodide. The amount of water titrated is proportional to the total current that is produced from this reaction. The water content can be determined from the current required for the titration. Validation of a method for coulometric determination involves evaluation of sample extraction time, sample size linearity, reproducibility ($n = 5$), and spike recovery over an appropriate range of percent water.



Documentation

All methodology is documented in a QTI analytical testing procedure (ATP). The document is created following method development and edited following method validation to include information such as solution stability and representative chromatograms. These documents are specific to each product and are the property of the contractor. Protocols for validation testing can also be generated upon client request.

Release and Stability Testing

QTI' s service offerings include storage facilities and resources to perform release and stability testing of comparative agents. Storage is available for all ICH conditions and special conditions that can be tailored to client requests. The laboratories are equipped with environmentally controlled chambers for storing samples under various temperatures and relative humidity. Samples are pulled at specific intervals and tested within 20 business days. Testing includes assay and impurities determination, dissolution testing, water content, and appearance. Stability protocols can be generated upon request. Result specifications are based on USP guidelines.

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