

STRENGTH AND STABILITY TESTING FOR COMPOUNDED PREPARATIONSⁱ

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ABSTRACT

Tests for strength are designed to determine how much of an active ingredient is in a sample. Stability tests are used to determine an expiration date of a product or a beyond-use date of a preparation. Being able to understand the difference between strength testing versus stability testing is the key to using the proper method to determine strength or stability. To determine strength, a method may or may not be stability indicating. When determining stability, the method must be stability-indicating. When using a stability-indicating method, both strength and stability can be determined. It is important that compounding practitioners understand the difference between strength and stability tests and how they are determined. Quality assurance programs are essential to establishing standards for compounded preparations.

INTRODUCTION

The terms “strength” and “potency” are often used interchangeably, with “potency” being used more by the general public and “strength” being used more by practitioners and within the official compendia. “What is the difference between strength (potency) and stability?” This seems like a rather simple question, and in some respects, it is. However, the cost of a full stability test for a formulation is considerably higher than that of a strength-overtime-test. To answer this question, one must understand the methods used to analyze the strength and stability of a compound.

The most common flaw in determining stability is failure to use an analytical method that has been demonstrated to be a stability-indicating method. The most important aspects of determining strength and stability are the methods used in the process. A stability-indicating method must be used to determine stability. Although stability-indicating methods have the capability of also determining strength, the reverse is not so—not all strength tests are capable of determining stability. The purpose of this communication is to explain the difference between strength and stability, why they are of importance, and how they are determined. The method used to determine the concentration of the active pharmaceutical ingredient (API) is the most critical step in the process and takes into account other variables, such as solubility, polymorphic forms, and others.

STRENGTH

Strength can be described as the concentration of the drug in a product or preparation. Strength tests are known as quantitative tests and are designed to determine how much of an API is in a sample. High-performance liquid chromatography (HPLC) is the typical methodology used in determining strength. HPLC is a preferred method because it is specific and efficient. Although HPLC can be used in stability-indicating methods, not all HPLC procedures are stability indicating—and they must not be assumed to be so.

Other methods used to test strength include titration, which uses the principles of chemistry, and microbial assays, which are sometimes used to test antibiotics. Titration is based upon a known chemical reaction with the desired drug. A microbial assay is performed by using bacteria and the antibiotic of choice and by examining the “zones of inhibition”. Ultraviolet (UV)-visible spectrophotometry also can be used to determine strength, but when used alone (without chromatography), UV-visible spectrophotometry can determine strength only for single analytes in solutions. Multiple compounds could interfere with UV absorption, resulting in erroneous results when UV-visible spectrophotometry is used alone. When performing a strength test, the methods used determine whether one will be able to determine stability as well.

The purpose of strength, or potency, testing is to establish or verify the concentration (strength, potency) of the API in the compounded preparation. USP has established that the acceptable range of most compounded preparations is typically $\pm 10\%$, or within the range of 90.0%–110.0%. The issue is that many “strength” tests do not separate the intact drug from the degradation products, and the degradation products show up under one peak in the chromatogram, thus giving the false information that the drug concentration has not changed, when it actually has. A stability-indicating assay, properly performed, will separate the degradation products/peaks and show the intact drug peak as it decreases in area or height, reflecting a change in the concentration of the intact drug.

STABILITY, INSTABILITY, AND INCOMPATIBILITY

Stability is the extent to which a product retains, within specified limits and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of its manufacture. The *United States Pharmacopeia 36/National Formulary 31 (USP 36/NF 31)*, in the table within general information chapter <1191> *Stability Considerations in Dispensing Practice*, provides definitions for five general types of stability:

- **Chemical:** Each active ingredient retains its chemical integrity and labeled potency, within the specified limits.
- **Physical:** The original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability, are retained.
- **Microbiological:** Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retain effectiveness within the specified limits.
- **Therapeutic:** The therapeutic effect remains unchanged.
- **Toxicological:** No significant increase in toxicity occurs.

Instability describes chemical reactions that are “...incessant, irreversible, and result in distinctly different chemical entities (degradation products) that can be both therapeutically inactive and possibly exhibit greater toxicity”.

Incompatibility is different from instability but must be considered in the overall stability evaluation of a preparation. Incompatibility generally refers to visually evident and “...physicochemical phenomena such as concentration-dependent precipitation and acid–base reactions, with the products of reaction manifested as a change in physical state, including protonation–deprotonation equilibria”.

Example

Some compounding practitioners have misconceptions about extending beyond-use dates, based for example on the notion of contracting with analytical laboratories to conduct a strength (potency) test that does not use stability-indicating methods, running assays at time 0, at 30 days, and at 60 days. Take for example a target concentration of the compound intended to be 10 mg/mL. The test result was one that indicated only strength, not stability, because the test did not use a stability-indicating method. In other words, at those predefined time points of day 0, 30 days, and 60 days, the lab analyzed only how much of the compound was present. The lab could not, however, differentiate the compound of interest from degradants or excipients in the preparation that may have been “co-eluting” in the chromatogram. The results might be reported that the compounded preparation was at a concentration of 10 mg/mL at each time point.

The results cannot be interpreted to determine a stability of 60 days, because in the analysis there could have been degradants or excipients that were present but not detected (again assuming that a stability-indicating method was not used in the analysis). To put it into numbers, the actual concentration of the active ingredient could have been 6 mg/mL, with 3 mg/mL of degradants and 1 mg/mL of excipients. The most important point to realize in this scenario is that strength but not stability can be determined, because stability-indicating methods were not used. Had stability-indicating methods been used to determine strength, then the results could have been used to determine a beyond-use date, otherwise referred to as stability. Using the previous example, if the concentration at time 60 days was 10 mg/mL and stability-indicating methods were used, one could be sure of looking at only the active ingredient.

Figure 1 represents a chromatogram of a nonstability-indicating HPLC method that can be used to quantitate the analyte of interest. *Figures 2* and *3* represent a chromatogram of a nonstability-indicating HPLC method containing analyte and degradant sample peaks that are not resolved. All that can be concluded is that there are degradants present in the sample at the time of the analysis. In *Figures 2* and *3*, no conclusions can be made about strength or stability. As for strength, the peaks are not resolved, which does not allow one to properly quantitate the analyte of interest. Stability cannot be determined, because stability-indicating methods were not used.

STABILITY TESTING

Stability testing includes method development, method validation, and a stability study. Method development will separate the active ingredient from its degradants and impurities, as well as any

other excipients in the preparation. This is done by force-degrading the active ingredient and inactive ingredients to ensure that no degradants are interfering with the analysis. In the process of forced degradation, high heat and humidity, UV radiation, acid exposure, base exposure, and peroxide exposure are performed on the compound. It is this step that is different from a simple strength test. *Figure 4* shows an example of a chromatogram of a stability-indicating HPLC method containing analyte and degradant peaks that are fully resolved from one another. When looking at this chromatogram, it is important to notice that the active ingredient, or analyte, is completely separated from its degradants and excipients. Stability can be determined from this type of study, because stability-indicating methods were used in the analysis.

The method validation confirms that the method meets certain criteria. The typical analytical characteristics used in method validation include accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and ruggedness, as outlined in general information chapter <1225> *Validation of Compendial Procedures*.

The stability study includes storing the preparation in stability chambers, testing the preparation at predetermined time points, and then determining its stability. These time points can be specified by the compounder or may be limited based on the particular compound. Once again, it is crucial to understand that the methods used to determine stability must be stability-indicating. Equally important to understand is that a strength test that uses stability-indicating methods can determine strength as well as stability.

HPLC DIODE-ARRAY DETECTORS

The PDA (photodiode array) detector is a device that scans from about 200 nm up to 400 nm in the UV range (and can reach 700 nm in the visible range in some instruments). The full array scans the eluent coming from the HPLC every second or so. The software starts at the beginning of a peak and makes scans (basically by “slicing” it into pieces) and then completes the scan instantly. The scans are compared (overlaid), and any change is identified. By using an algorithm, the software calculates the “peak purity” by comparing the middle peak scans with those of the leading and trailing tails. If the scans overlay perfectly, then the peak purity will be 100%. If the scans do not overlay perfectly, then the result is a calculated percentage. The issue with this approach is that a UV scan is not necessarily specific, and small changes in a drug molecule can occur that may not be detected by the scan but may alter the drug strength, although based on the assay, the strength may not have changed. The molecule contains “chromophores” that absorb the UV light at different wavelengths and efficiencies. If a molecule degrades but the change is not in a strong chromophore, then the change will not appear in the scan, and the strength will not be determined accurately.

Peak purity evaluation should be performed during validation as part of the specificity test of the forced-degradation samples. The peak purity test helps to ensure that the method can separate degradation products during a stability study, and “strength” of the API can be assessed versus the reference standard. One can apply peak purity analysis to compounded preparations for routine strength testing and maybe time point testing, as part of the beyond-use date of the compounded preparations. But the method itself still needs to be validated to become a standard monograph method. The PDA method for peak purity determination can be used to “supplement or support” a stability-indicating analytical method but should not be used in place of it.

SUMMARY

In summary, the practitioner who extemporaneously compounds must ensure the strength, quality, identity, and purity of compounded preparations. An outsourced analytical laboratory can assist by providing quality control and quality assurance. Determination of strength or concentration is invaluable in maintaining good preparations that are accurate and precise. A stability-indicating method must be used to determine the beyond-use date of a compounded preparation.

FIGURES¹

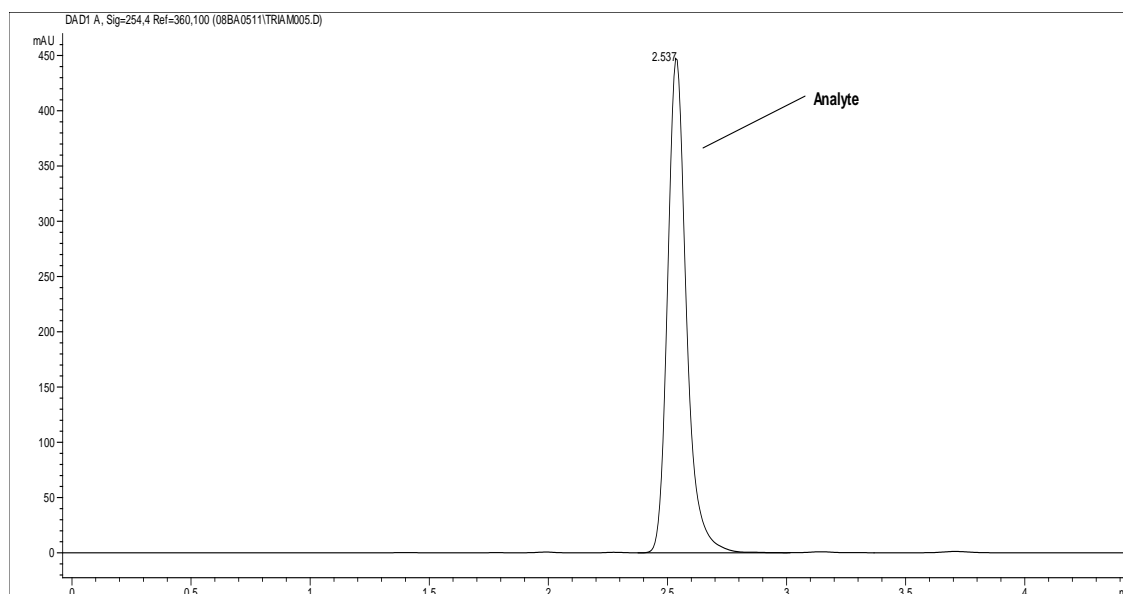


Figure 1. An example chromatogram of a nonstability-indicating HPLC method that evaluates the potency of a single analyte.

¹ Figures reproduced with permission from Kupiec TC, Skinner R, Lanier L. Stability Versus Potency Testing: The Madness is in the Method. Int J Pharm Compd. 2008 Jan/Feb; 12(1): 50-53.

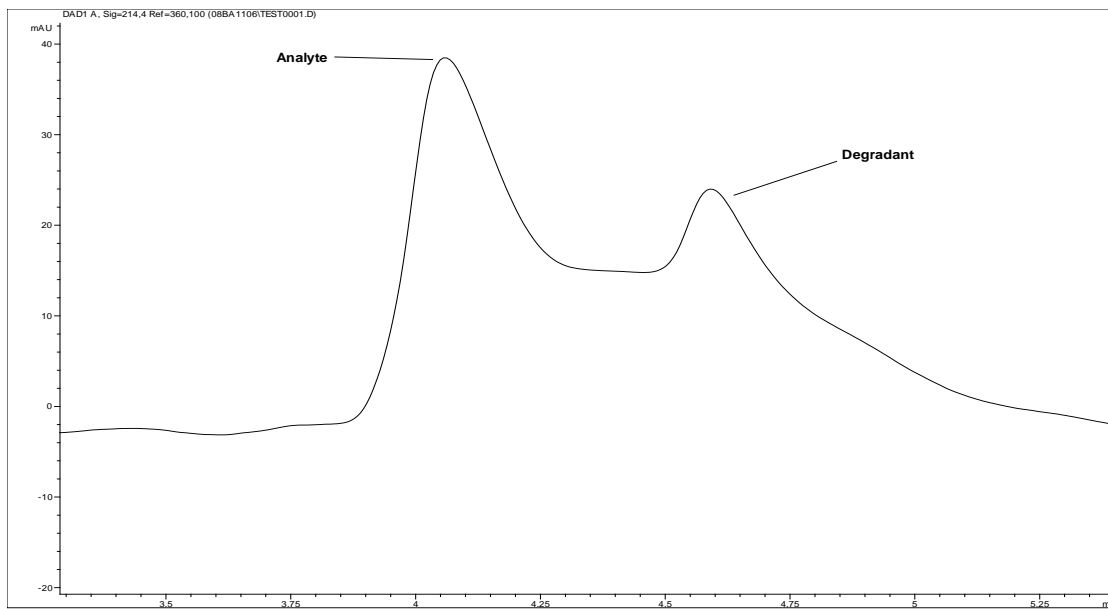


Figure 2. An example chromatogram of a nonstability-indicating HPLC method that evaluates the analyte and degradant sample peaks.

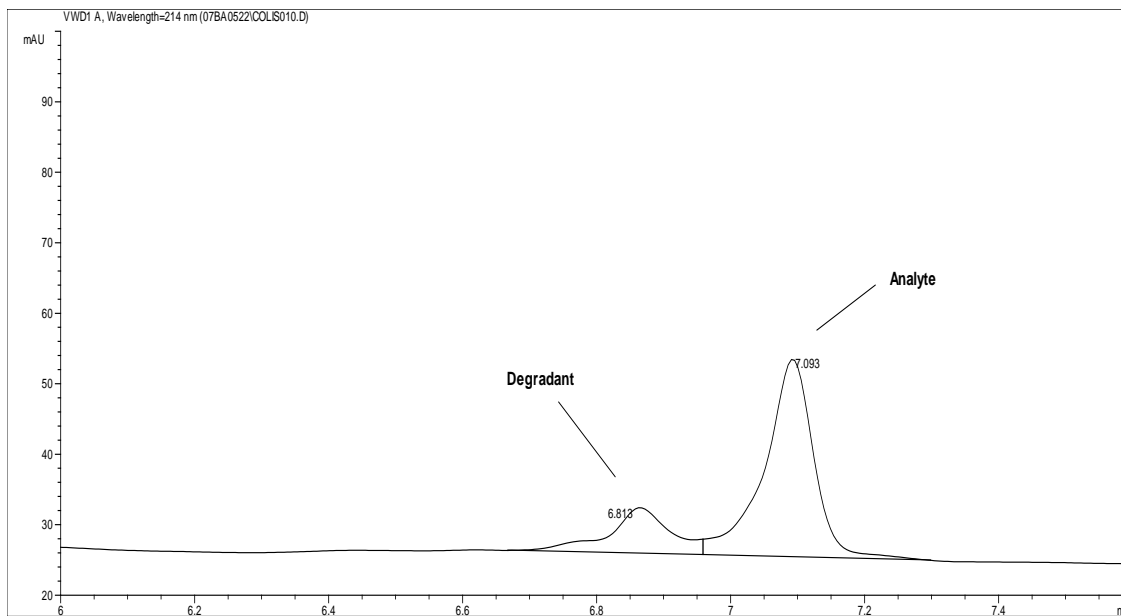


Figure 3. An example chromatogram of a nonstability-indicating HPLC method that evaluates the analyte and degradant peaks that are not fully resolved from one another.

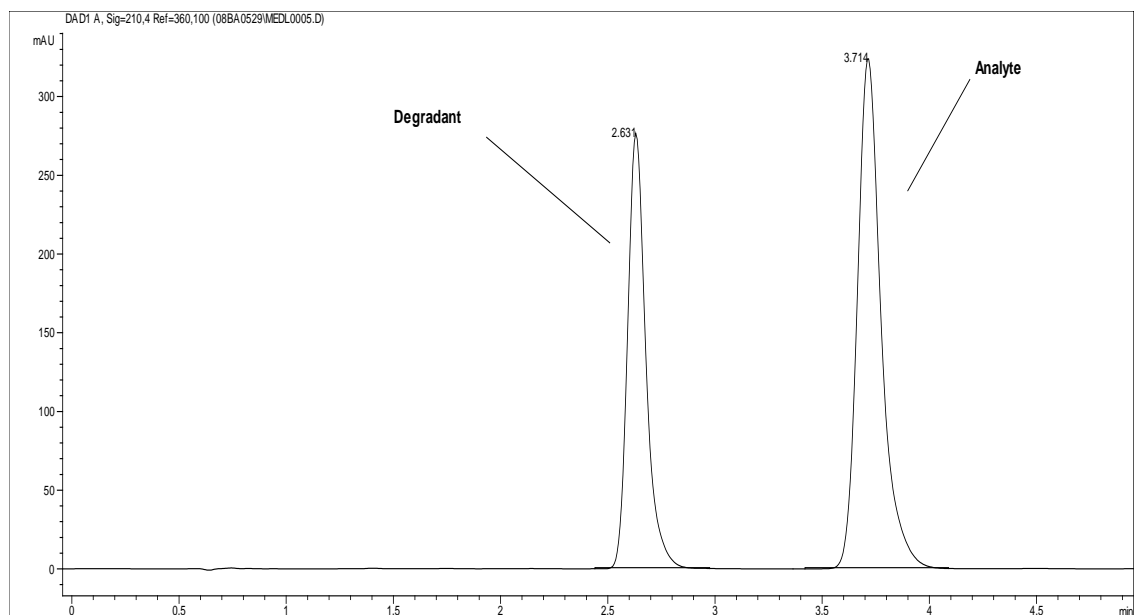


Figure 4. An example chromatogram of a stability-indicating HPLC method that evaluates the analyte and degradant peaks that are fully resolved from one another.

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