

Stability studies: Five years later

LAWRENCE A. TRISSELL AND KARL P. FLORA
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It has been five years since the editorial "Avoiding Common Flaws in Stability and Compatibility Studies of Injectable Drugs"¹ appeared in the *American Journal of Hospital Pharmacy*. In the ensuing period, it is our perception that important and noteworthy improvements in reports of such studies have become the standard rather than the exception. Nevertheless, there is still substantial room for improvement.

The 1983 editorial outlined five common deficiencies frequently seen in articles appearing for publication consideration and called for their rectification. Briefly, these five common flaws were

1. Lack of a complete description of the materials, test conditions, and methods;
2. Failure to use a stability-indicating analytical technique;
3. Failure to perform an analytical determination at the outset;
4. Use of inadequate numbers of test samples and replicate assays; and
5. Conclusions that overreach or otherwise fail to fit the results.

In 1987, articles appearing for publication consideration in pharmacy journals were generally quite thorough in describing the materials, methods, and test conditions. Very few failed to perform a time-zero analysis. Replicate assays of multiple test solution samples are common in study designs today, although occasional papers still report the results of a single determination of a single test solution. Authors do appear to be doing better at drawing appropriate and supportable conclusions from the data they derive.

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Even with these improvements, there remains a recurrent problem—the frequent use of analytical methods for which validation of the stability-indicating capability is either inadequate or nonexistent. This was the most common flaw in 1983, and it remains so today. Important improvements have certainly been made; excellent stability articles now appear with regularity in the pharmacy journals. Nevertheless, too many articles lacking adequate method validation are submitted for publication and even find their way into print. Strangely, this happens despite a general agreement with the basic premise that the analytical method should accurately quantitate the intact drug or drugs in the presence of decomposition products and other solution components. Compliance with this premise is obviously essential to produce accurate results. A method that fails to meet this criterion yields results that are suspect, if not simply wrong.

The argument is not with the premise but, rather, with the proof. To assure oneself and, ultimately, the reader of the paper that the criterion is met, an author must prove that the method performs adequately. This is not very difficult to do if the method is, in fact, stability indicating. Usually, validation of a method is performed in one of two ways. Fresh intact drug can be subjected to extremes of pH and intensive heating to intentionally decompose it. Alternatively, a solution of the intact drug can be spiked with known decomposition products. A stability-indicating method will accurately and selectively quantitate intact drug in the presence of decomposition products and other solution components.

So the question becomes whether this extra work of validation is really necessary. With the advent of modern analytical technology, why go to this trouble? Simply stated, validation is an essential step no matter what the analytical method. In theory, any method that meets the requirements can be used. In practice, high-performance liquid chromatography (HPLC) is frequently used and may be the method of choice for stability studies because of its inherent advantages that often lead to stability-indicating techniques. Also, the various "binding" assay techniques that are familiar and readily

available in hospital laboratories have been used often in stability studies. Such binding assays include RIA (radioimmunoassay), EMIT (enzyme-multiplied immunoassay technique), ELISA (enzyme-linked immunosorbent assay), and TDX (a fluorescence polarization immunoassay).

But not all HPLC procedures are stability indicating, and they must not be assumed to be so. Things that can and do go wrong with HPLC analysis include decomposition products eluting with the intact drug, other drugs or solution components interfering with or eluting with the intact drug, and inappropriate or mistaken sample preparation leading to erroneous results. Similarly, one cannot assume that the binding methods are stability indicating; they can cross-react with related compounds. A couple of examples will illustrate these kinds of problems.

Ray et al.² developed an HPLC method intended to determine the stability of Δ^9 -tetrahydrocannabinol (THC) capsules. The method gave a single symmetrical peak for THC that was nicely separated from several known decomposition products and isomers (Figure 1). However, during storage under stressed conditions (37°C) the width of the THC peak increased slightly. Although it was tempting to discount this variation as trivial, further work using simultaneous monitoring at two wave lengths showed inconsistencies that suggested the presence of an unresolved peak. In fact, a decomposition product was found that eluted with the intact THC peak, making the drug appear to be more stable than it really is. Subsequently, an HPLC technique using two columns was developed to provide a truly stability-indicating method (Figure 2).

Figure 1. HPLC chromatogram from a Δ^9 -THC capsule stored at 37°C for 24 months with the initial system. Conditions: Altex Ultrasphere 5- μ m column; mobile phase, acetonitrile: 1% acetic acid (70:30, v/v); flow rate, 1.0 mL/min. Reprinted with permission

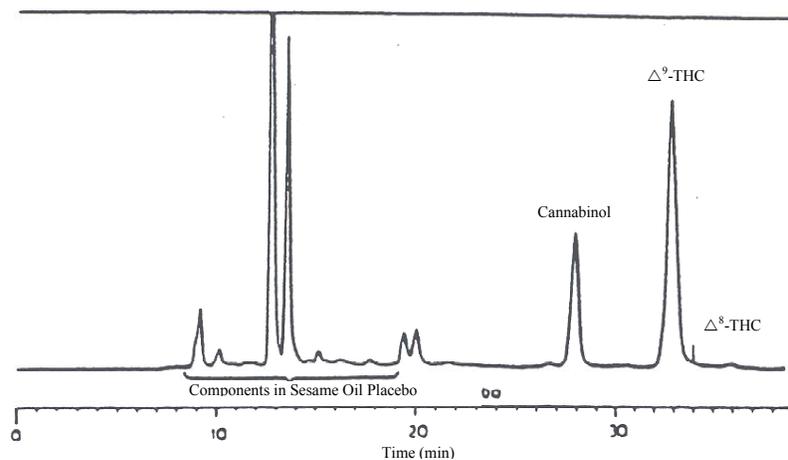
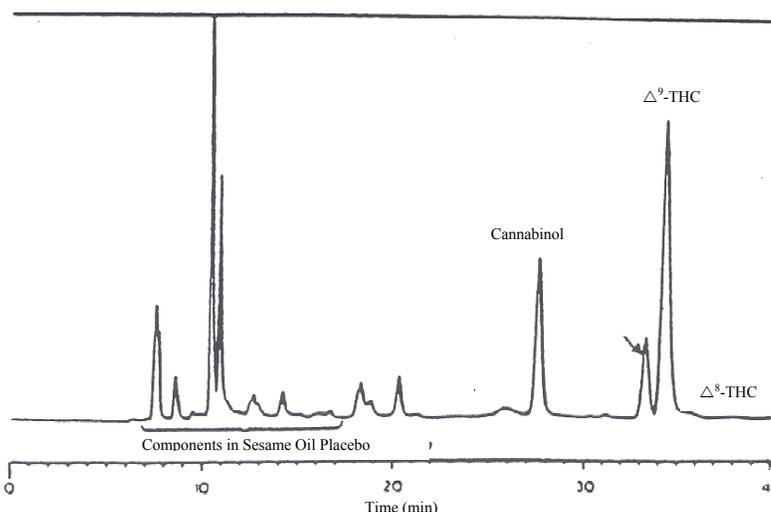


Figure 2. HPLC chromatogram with the modified system, showing complete separation of the Δ^9 -THC and the impurity peak. Conditions: Altex Ultrasphere 5- μ m column in series with an Altech Spherisorb 3- μ m column; mobile phase, acetonitrile: 1% acetic acid (85:15, v/v); flow rate, 0.5 mL/min. Reprinted with permission



AJHP policy on manuscripts dealing with drug stability

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Use of the popular immunoassay assays can also lead to erroneous results. The selectivity of these methods is imparted by the selectivity of an antibody to bind to the drug being measured. However, the antibody may also bind (cross-react) with related chemical entities. Most of these methods have been optimized for selectivity to a drug in the presence of metabolites in biological fluids. Metabolites and decomposition products are not necessarily the same. Furthermore, decomposition products are sometimes more closely related chemically to the parent drug than are the metabolites.

Bastos and Hoffman³ examined the specificity of an EMIT assay designed for amphetamine. They found that many similar compounds cross-react with the antibody. For example, phenethylamine, which is related chemically to amphetamine, cross-reacted with the antibody. A 3.1 µg/mL phenethylamine sample assayed as 0.32 µg/mL of amphetamine. The binding may have been selective, but it was not totally specific. Even worse, phenmetrazine 0.95 µg/mL assayed as 1.05 µg/mL of amphetamine. If an antibody being used in a stability study binds to decomposition products or other drugs in an admixture, quite clearly the results may be erroneous.

To reiterate, no matter which analytical method is being used, it is essential that the stability-indicating capability of the assay be verified.

The accumulated body of stability literature is replete with studies in which the methods were not determined to be stability indicating. Nevertheless, practitioners often have had to use the information in their practices for lack of any other data. We cannot fault those ground-breakers of the past who worked, erred, learned, and worked harder yet, eventually leading us to a much more scientific approach to stability determination. But in 1988 and beyond, validation is key; to do less is unacceptable. Practitioners who use the data and the patients who are the ultimate beneficiaries have the right to expect meaningful, accurate, validated studies.

References

1. Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am J Hosp Pharm.* 1983; 40:1159-60. Editorial.
2. Ray G, Crook M, West N et al. Comparison of the analysis of Δ^9 -tetrahydrocannabinol capsules by high-performance liquid chromatography and capillary gas chromatography. *J Chromatography.* 1984; 317:455-62.
3. Bastos ML, Hoffman DB. Comparison of methods for detection of amphetamines, cocaine and metabolites. *J Chromatogr Sci.* 1974; 12:269-80.

Studies evaluating drug stability and compatibility continue to fill a substantial number of pages in *AJHP*. In the past few years the quality of these papers has improved dramatically, reflecting the use of more accurate and specific analytical techniques by a larger proportion of authors.

In keeping with the advances in this area, *AJHP* has gradually adopted more stringent requirements for proof of drug stability, based on advice we have received from experienced reviewers. Given the imperfect nature of the peer-review process and legitimate differences of opinion among some reviewers about exactly what constitutes "acceptable" proof of stability, the editors must often arbitrate the fate of individual stability papers about which reviewers disagree in their recommendations. In making these decisions we must rely on our collective experience in evaluating hundreds of such studies and reviewers' critiques in an attempt to ensure that all papers meet certain minimum requirements for adequate documentation of drug stability.

The guidelines published in an editorial by Trissel¹ in 1983 continue to serve as the basis for our evaluation criteria for stability studies; all reviewers of such studies are sent a copy of that editorial. Authors planning to submit manuscripts on drug stability to *AJHP* should review these guidelines, which are referred to in "Procedures for submission of manuscripts to journals published by the American Society of Hospital Pharmacists" (page 177, January 1988 issue). The commentary by Trissel and Flora in this issue² further details the need for using appropriate methods of proving drug stability.

All of the major components of a good stability study mentioned by Trissel—complete description of materials, test conditions, and methods; use of a stability-indicating assay; performance of a time zero determination of drug concentration; use of replicate assays; and an appropriate conclusion based on the results—are considered in our review of these papers.

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Unfortunately, we still receive papers from authors who have not followed this advice. Probably the most common omission in these studies is a description of the procedures used to prove that the assay or assays used can distinguish between the parent drug, degradation products, and other components of the admixture. In most cases authors should perform these validation procedures using the specific conditions of their current study, rather than attempting to reference another article in which the stability-indicating nature of the assay was confirmed under similar but not identical conditions. Most of our advisors concur with this recommendation and insist that such validation procedures do not involve substantially more effort than would otherwise be required for performing these assays. Also, when authors believe it is acceptable to use a modification of a published assay, they should describe exactly what modifications to that assay were made (e.g., changes in the mobile phase or detection wave length) so the reviewers and editors can evaluate the appropriateness of such changes.

Authors interested in submitting stability studies to *AJHP* may wish to consider validating previously published drug stability studies in which the stability-indicating nature of the assay was poorly documented. In those instances we will give priority to studies involving drugs with known stability problems or drug combinations that are frequently encountered in the patient-care setting.

Studies dealing solely with the visual compatibility of a drug or drugs have a place in *AJHP* if few or no stability data are available elsewhere and the drug(s) and diluents in question are likely to be used together frequently in practice. These studies provide no more than a gross evaluation of the physical characteristics of an admixture (i.e., presence of turbidity or a precipitate) and are published as Notes or Letters to the Editor.

By promoting stricter standards for drug stability in published papers, we are helping achieve the intended purpose of these studies; i.e., to provide practical, reliable information that pharmacists can use in the patient-care setting. As researchers and practitioners who set high standards for themselves and their profession, pharmacists should continue to strive for improved reliability and accuracy in studies of drug stability when the methods exist to accomplish this.

References

1. Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am J Hosp Pharm* 1983;40:1159-60. Editorial.
2. Trissel LA. Flora KP. Stability studies: five years later *Am J Hosp Pharm*. 1988; 45:1569-71. Commentary