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PRACTICAL CONSIDERATIONS FOR COMPENDIAL METHOD SELECTION

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INTRODUCTION AND PERSPECTIVE

Analytical methods are an integral part of the compendial effort to establish meaningful drug standards. A wide range of instrumentation and analytical techniques are employed in USP/NF methods, and continuing advances in analytical technology continue to increase the available alternatives. In choosing a method, accuracy, reproducibility, and ruggedness are important considerations. Specificity and sensitivity are also important. But then, it could be said that these are desirable characteristics for many analytical methods. More specifically, what are the special circumstances which surround the use of compendial methods and therefore exert a strong influence on method choice?

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The need for specificity takes on a new meaning when it is considered that USP/NF methods are to be used for samples on which there is limited knowledge of composition and past history. (The manufacturer is much better informed of this). What is known is the labeling claim that it is a specific article, with the attendant features of strength, quality, and purity.

There is often a limited knowledge of the process and its impurities. To exhibit specificity, a method may need to separate several sets of process impurities. Likewise, there is often limited knowledge about the formulation. What excipients, preservatives and flavors are present which interfere? A method must often be suitable for several formulations and several sets of added substances.

Examples of this may be found in the analysis of barbiturate elixirs. In addition to propylene glycol, parabens or benzoic acid may be used as preservatives in butabarbital sodium elixir. The presence of propylene glycol presents a minimal interference or sample preparation problem. Benzoic acid can be extracted from butabarbital simply by pKa differences, but if parabens are present, a more elaborate method is necessary. Separation of parabens from butabarbital can be a problem even with column chromatography[1] and GLC[2]. Selective hydrolysis[1] or selective bromination[2] of parabens followed by chromatography are techniques that have been proposed to achieve method specificity.

A second example of interference by additives is shown by the analysis of commercial phenobarbital elixirs using direct

HPLC injection, a microparticulate C18 column, and a methanol - pH 3.5 mobile phase. This system separates predicted additives (saccharin, parabens) and is suitable for solid dosage forms. Due to interferences, only one of three elixirs could be analyzed without prior extraction of the phenobarbital with chloroform. In this instance direct injection may be suitable for one manufacturer's product, but not for a compendial method. USP methods, therefore, may need more steps and may need to be more involved than in-house methods.

Method reproducibility and ruggedness are important considerations in compendial method selection.[3] The General Notices of the USP[4] states that alternative methods may be used to demonstrate compliance, but in the case of a dispute, compendial methods are the final judge of product integrity. The procedures must therefore be workable in a number of laboratories, both industrial and governmental, both purchaser and vendor. Between-lab reproducibility is just as important as within-lab reproducibility.

The equipment, columns, and reagents necessary for compendial tests must be readily available as opposed to the use of specialty or home-made equipment. A separation which depends on one brand of TLC plates or an HPLC column of size or type which is not routinely available may be suitable for an in-house method but would not be preferred for a compendial method.

A general policy is that no single manufacturer be specified for reagent or equipment items except when

absolutely necessary. It is impossible to predict that one source will continue to be of better quality or more permanent than all others. Since it takes time to replace a compendial method, it is unwise to base a method on a single source which may be here today and gone tomorrow.

The problem of column reproducibility is familiar to anyone working with chromatographic methods. It is less pronounced with GLC methods, but relative to GLC, HPLC is in a developmental stage, and variation in column packings is still a common occurence. Problems have been minimized by the following procedures: (1) use of several columns in method development so that methods are not based on exceptional separations which cannot be reproduced; (2) use of system suitability tests[5,6] which specify the minimum resolution factors, minimum tailing factors, and multiple injection precision necessary for the analysis; and (3) increased reliance on collaborative studies which may detect problem areas in unreproducible methods. Consideration of comments based on collaborators' experience helps to refine the method and may add another order of magnitude to its universality.

Method cost and work-intensity are always subjects of interest and should be mentioned in a discussion of practical aspects of method selection. While other factors are more important, these factors are considered in compendial method choice. For instance, the USP does not require a $150,000 mass spectrometer for an identity test when an infrared spectrum gives the neces-

sary information. On the other hand, precision, specificity, and sensitivity cannot be sacrificed just to prevent adoption of expensive methods. In some cases, two methods are essentially equivalent, and the less work-intensive procedure can be chosen. Several examples of this are mentioned in the following discussion of specific methods and their alternatives.

EXAMPLES OF METHOD SELECTION

Once a perspective on compendial needs is obtained, one must consider the particular requirements for each drug and dosage form and the general requirements for each type of monograph test. In addition, no individual test stands alone--its suitability depends on the presence or absence of other tests. For instance, it is preferred that monographs taken as a whole be stability-indicating. This can be achieved by a specific assay or, in its absence, by limit tests for decomposition products.

Limit Tests

Limit tests are often required in a monograph. Limit tests involve either a choice between an initial separation step or the measurement of small amounts of impurities in the presence of the drug. Choice of both the separation and detection procedures are dependent on the nature of the drug and its impurities.

The USP limit test for propoxyphene impurities[7] limits the acetoxy analog, a process impurity, and the free carbinol, both a process impurity and principal degradation product. This GLC limit test complements the assay, a nonaqueous titration,

which is not, and need not be, stability-indicating. The GLC procedure is attractive because it detects the impurities at the 0.5% level without prior separation. TLC, a possible alternative, is less desirable because of plate overload problems and insensitive detection. IR and melting point determinations also lack the necessary sensitivity.

A proposed GLC limit test for p-chloroacetanilide[8], a process impurity in acetaminophen, uses a partition column for prior separation. HPLC was considered as a possibility for the detection step when the method was developed, but several different C18 columns gave widely different retention times. GLC on a 3% polyamide column (Poly A-103 or equivalent) was considered to be more reliable than either HPLC or a previous TLC procedure.[9]

TLC has found considerable use in compendial limit tests, particularly in steroid monographs. The limit tests for estradiol in estradiol valerate and chromatographic impurities in norethindrone are two examples. TLC limit tests are often desirable since efficient separations and high sensitivity can often be obtained with a minimum of equipment.

A Bratton-Marshall colorimetric procedure is used to limit diazotizable impurities in thiazides[10]. This is an example of a non-chromatographic limit test which has the advantages of sensitivity, selectivity, and adaptability to automation.

Assays

A complete range of assay methods is now found in the compendia, but the trend is clearly toward more chromatographic

methods, both for stability indication and because of the ease in which they solve interference problems. Among chromatographic methods, HPLC is gradually replacing TLC and column methods which tend to be work-intensive. GLC will continue to find usefulness and is often preferred to HPLC.

An HPLC method[5] was chosen for the USP assay for progesterone injection. This is a case in which the dosage form will affect the method chosen--the oil-based injection contains a number of ingredients which could interfere with a procedure. This method replaced a 2,4-dinitrophenylhydrazone gravimetric procedure which is unspecific and imprecise. Specificity could be obtained by GLC, but degradation during analysis is a problem. Another possibility is a partition column followed by UV detection[11], but the HPLC procedure combines speed with specificity.

A Bratton-Marshall colorimetric determination after potassium permanganate oxidation was formerly the official assay for folic acid. However, some lots of raw material contain unidentified impurities which, like folic acid, give the color reaction only after oxidation. In addition, precision problems were so considerable that inconclusive results were obtained by collaborators on the suitability of a new lot of reference standard. These problems were solved by the development of new HPLC and TLC methods[12] which gave equivalent separation of impurities and degradation products. HPLC is preferred since quantitative TLC is more laborious and time-consuming. Two samples of folic acid were analyzed by fourteen collaborators in a collaborative study

of the HPLC method. Inter-lab variation was less than 2%.

The USP sorbitol assay is a GLC method[13] which employs derivatization with n-butyl boronic acid. It replaces a laborious column titration method. Chromatographic methods employing derivatization are not preferred if other methods are available. However, sorbitol is too polar for GLC, and has no UV for HPLC. It is relatively lacking in analytical handles.

A proposed GLC method for barbiturates[2] using a polyamide column (Poly A-103) has been studied collaboratively. A 0.9-m x 4-mm column at 200° was used. UV methods have been used for barbiturates, but barbiturates may contain UV absorbing impurities and dosage forms often contain interferences. This is a case where selection of the column itself is an important part of method choice. A number of GLC phases have been used, but most not very successfully due to polarity of the barbiturates. An important characteristic of the polyamide column is that it gives symmetrical peaks and low non-linear adsorbance[14] even after considerable use.

Secobarbital Elixir, Pentobarbital Capsules, and Amobarbital Tablets were analyzed by the GLC procedure in a collaborative study. The eight collaborators reported a system suitability multiple injection variability of less than 1.5%, and tailing factors were less than 1.5. Interlab variation in assay values was less than 3.2% in all cases and less than 2% for a majority of the samples.

Iodochlorhydroxyquin may often be contaminated by several isomers. It has low solubility in most solvents and is hard to

deal with chromatographically. Not all isomers are separated by TLC[15]; with HPLC a large amount of tailing is exhibited in most systems. A GLC method using silyl derivatization[16] has the advantage of sensitivity and specificity, but stability of the derivative can be a problem. The present USP method, an IR assay with quantitation of the band at 14.4 μ,[15] is specific in the presence of anticipated impurities.

Sodium and calcium dioctylsulfosuccinate are examples of compounds for which no ideal method is presently available. They too have few analytical handles. They are too polar for GLC and lack UV absorption. Quantitation by IR is nonspecific. TLC is suitable for identification purposes, but quantitation is a problem due to lack of functional groups. Official methods now employ ion-pair titrations and visible determination of ion-pair complexes. These methods are sometimes subject to interferences by formulation ingredients, precision problems, and emulsion formation.

Identity Tests

The purpose of identity tests is given in the General Notices of the USP.[17] They are provided as an aid to verify the identity of labelled substances, but do not necessarily establish proof of identity. They are complimented by other monograph tests. Identity tests should be simple, yet specific. IR is usually the most useful and is the most used; when the whole spectrum is taken into account, considerable specificity is obtained. TLC is often useful, especially when dis-

tinguishing the compound from a series of closely related compounds.

Content Uniformity/Dissolution

In the case of content uniformity and dissolution tests, specificity is secondary to speed and precision. These are areas in which automated methods prove useful. Official methods may be automated and used as alternative methods. An example is the content uniformity for nitroglycerin tablets given in the General Information section of the USP.[18] This automated method uses the same basic chemistry as the manual official method. Official automated methods may be acceptable for high volume products. An automated content uniformity test for all three components of hydralazine, hydrochlorothiazide, and reserpine tablets has appeared in USP XX Comment Proof.[19]

CONCLUSION

In the preceding discussion I have mentioned a number of factors that need to be taken into consideration when choosing a compendial method. Compromise is often required and the ideal is rarely obtained. Nevertheless, consideration of these factors will help to provide methods which are reproducible from lab to lab, specific, yet not unduly burdensome. Such an approach will play an important part in ensuring pharmaceutical quality.

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