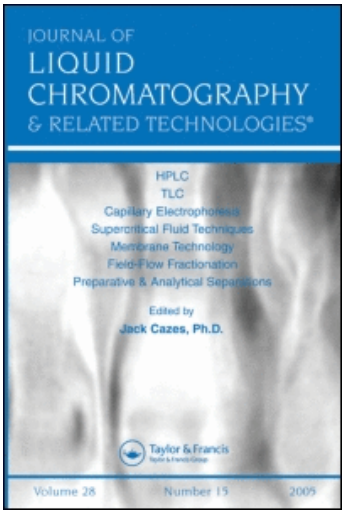

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Chromatographic Method Validation: A Review of Current Practices and Procedures. I. General Concepts and Guidelines

Dennis R. Jenke[a]
[a] Baxter Healthcare Corporation, William B. Graham Science Center, Round Lake, IL

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CHROMATOGRAPHIC METHOD VALIDATION: A REVIEW OF CURRENT PRACTICES AND PROCEDURES. I. GENERAL CONCEPTS AND GUIDELINES

Dennis R. Jenke
Baxter Healthcare Corporation
William B. Graham Science Center
Round Lake, IL 60073

ABSTRACT

Validation of analytical methodologies is widely recognized as an important aspect of the development/utilization of analytical procedures and is widely required in support of product registration applications. Detailed, specific and comprehensive guidelines for the performance of analytical validations are not universally available. In this manuscript, the role and concept of validation is defined, the necessity for validation is established and published guidelines related to appropriate validation parameters are reviewed. The validation of chromatographic methods for pharmaceutical applications is particularly emphasized.

INTRODUCTION

Chromatographic methods are commonly used for the quantitative and qualitative analysis of environmental and pharmaceutical samples. The object of the analysis is to generate reliable, accurate and interpretable information about the sample. In order to ensure that the analytical method fulfills this objective, it undergoes an evaluation loosely termed validation. Such a

validation is necessary especially in trade, in regulatory control and in cases of dispute wherein the results of the chemical analysis must be unambiguous and interpretable in only one way.

While the need to validate analytical methods is clear, the mechanics of performing a rigorous validation are not generally well defined. Questions of interest include:

* which validation parameters should be utilized,

* what specific procedures should be used to evaluate a particular parameter, and

* what is the appropriate acceptance criteria for a particular parameter.

Two factors contribute to the uncertainty associated with defining an effective validation protocol. Firstly, the general class of chromatographic methods is sufficiently broad and the applications of the procedure are sufficiently diverse that rigorous, effective, practical and defensible validation protocols are generally technique and application specific. Secondly, while guidelines exist for general classes of applications (e.g., references 1-8), they "are very often vague, sometimes quite inaccurate and misleading and rarely provide the development analyst with guidance on what really should be required of a validation exercise."[9]

In order to "get a handle" on the current state of thinking on the general topic of analytical method validation, a literature review was performed. The result of that review are summarized in a three part series of articles of which this is the first. These articles focus on the following:

* Part I; defining validation, establishing the need for validation, and identifying significant validation parameters.

* Part II: defining, identifying procedures for and summarizing acceptance criteria for specific significant validation parameters.

* Part III; defining, identifying procedures for and summarizing acceptance criteria for secondary validation parameters and related topics (e.g., re-validation and system suitability).

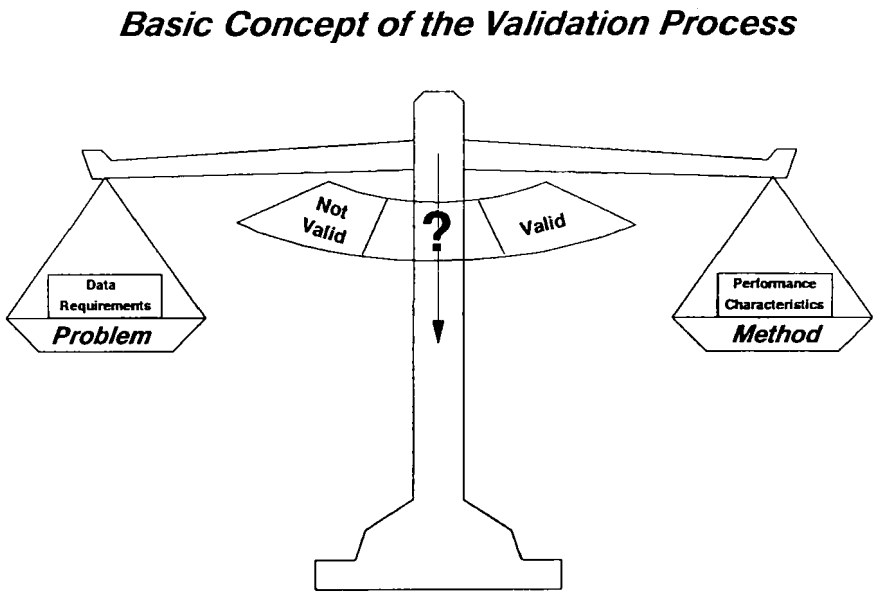

Figure 1. The Basic Concept of Method Validation wherein performance data are compared to pre-determined data requirements to assess whether the method is valid. Concepts suggested by J.K. Taylor (reference 48).

THE REGULATORY PERSPECTIVE ON VALIDATION

Test procedures for the assessment of the quality levels of pharmaceutical products are subject to various requirements. In the United States, the Current Good Manufacturing Practice (cGMP) regulations require that test methods, which are used for assessing compliance of pharmaceutical products with established specifications, must meet proper standards of accuracy and reliability.[2] Pertinent sections in the Code of Federal Regulations (21 CFR, ref. 10) include:

Section 211.165(e): The accuracy, sensitivity, specificity and reproducibility of test methods employed by the firm shall be established and documented.

Section 211.166(e.3): (The written program shall be followed and shall include) ... reliable, meaningful and specific test methods.

Section 211.194(a.2): The statement shall include the location of data

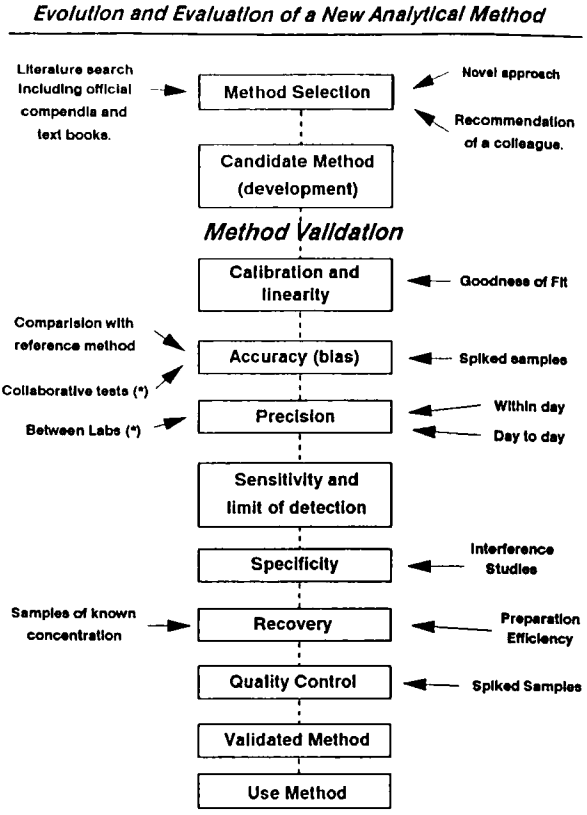

Figure 2. The Evolution and Evaluation of an Analytical Method as suggested by A.C. Mehta (reference 18). After the method has been conceived and operationally developed, its performance is validated with respect to several different performance parameters. Successful completion of the validation results in a method which can reliably be used to characterize "real" samples.

that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. The suitability of all testing methods shall be verified under actual conditions of use.

Additionally, new product applications (NDA, ANDA, IND) submitted to the federal regulatory agency (FDA) must include method validation data. FDA validation guidelines include those indicated in the CFR regulations as

well as an evaluation of method ruggedness. To wit, "the purpose of validating NDA methodology in FDA laboratories is to make certain that a competent analyst can use the applicant's procedures on the agencies equipment and obtain results that are comparable to those submitted with the NDA."[11]

GENERAL VALIDATION CONCEPTS

Several references provide a working definition of a valid method. These include that the valid method:

* is suitable (reliable) for its intended use;[2,8,12,16,29,30,33,35]

* provides useful analytical data in a specific situation;[15,33,47,48]

* meets the pre-determined requirements (specifications) of the analytical problem;[2,15,16,30,34,48]

* has an established level of performance [accuracy, consistency, reliability];[9,12,13,14]

* does what it is supposed (expected) to do.[9,28]

The term validation thus is relative in the sense that it implies an activity of demonstrating that the process or procedure under examination accomplishes what is claimed or intended. As shown in Figure 1, then, the validation process becomes the action of comparing behavior observed under rigorously defined conditions with pre-determined performance expectations. The extent to which the observed performance agrees with the expectations determines whether the process is valid or not. Additionally, it is clear that the specifics of the validation (which parameters, what procedure, what requirements) are application dependant since the intent of the assay and its performance claims are themselves application specific.

The relationship between validation and the other phases of the method evaluation/utilization process is often unclear. The hierarchical concept of method utilization, wherein the stages of method development, validation and utilization are distinct and sequential (e.g., Figure 2) represents an situation which is practically undesirable and potentailly inefficient. Method development without some rudimentary method validation activities can readily lead to a "vicious cycle" wherein promising methods are subjected to rigorous

Type of Validation Data Reported

(From a Survey of Published LC Analysis of Drug Substances and Dosage Forms)

Parameter		Number of Occurances		
		Tablet and Capsules [a]	Solid and Liquids [a]	Liquids
Accuracy	Standard Add.	2	2	2
	Spike, 1 level	11	4	2
	Spike, 2-3 levels	6	4	8
	Spike, >3 levels	8	5	1
	Alt. Method	13	3	3
Precision	Repeatability	17	3	5
	Reproducibility	29	17	7
Linearity		34	12	7
Minimum Quantifiable Level		19	5	2
Specificity	Deg. Products	15	9	7
	Deg. Kinetics	10	10	0
	Forced Degr.	7	7	7
	Analogs	7	3	4
	Impurities	3	1	0
Quantitation	Areas	20	7	11
	Heights	12	10	4
	Internal Std.	16	12	4
System Suitability		18	5	5
Number of References		47	22	15

Figure 3. Type of Validation Data Reported by T.D. Wilson as a result of a 1990 review of published liquid chromatography methods used in the evaluation of pharmaceutical samples (reference 28).

validation protocols only to fail one or more criteria and thus require additional development and re-validation. Thus several authors propose that validation be a two stage process, with rudimentary or pre-validation activities occurring during development and a formal validation assessment occurring after the method development process has been completed.[31,39,45] In this way, the validation specialist has some assurance going into the formal validation study that in fact the validation results will be favorable.

Test Type	Number of Responses Indicating that this Validation Parameter was Used for a Particular Test (maximum is 20)									
	Accuracy	Precision (1)	Precision (2)	Linearity	Sensitivity	Selectivity	LOD	LOQ	Solution Stability	Ruggedness
ID Tests (Specification Tests)										
GC, LC, TLC, CE	0	0	0	0	0	5	1	0	1	1
Related Substances Tests (Specification Tests)										
TLC	14	7	9	9	3	16	16	11	10	9
GC	16	14	8	16	6	14	14	13	12	9
HPLC	19	16	13	19	8	20	18	17	15	12
CE	3	3	3	3	1	3	3	3	3	3
IC	1	1	0	1	1	1	1	1	1	0
Various Assays (Specification Tests)										
GC, LC, TLC, CE	18	17	15	18	10	17	8	8	16	14
Degradation Products (Stability Tests)										
TLC	14	8	8	9	3	14	16	15	11	8
GC	14	11	6	14	6	11	12	11	11	8
HPLC	16	15	12	16	9	17	17	15	16	12
CZE, CE	1	1	1	1	1	1	1	1	1	1

(1) Repeatability (2) Reproducibility

Figure 4. Number of Responses Indicating that a Validation Parameter is Applied to a Test on Bulk Active Ingredient/Synthesis Material at Various Stages in the Product Development Stage. Data is from a Survey of UK Pharmaceutical Companies by G.S. Clark.[49]

Test Type	Number of Responses Indicating that this Validation Parameter was Used for a Particular Test (maximum is 20)									
	Accuracy	Precision (1)	Precision (2)	Linearity	Sensitivity	Selectivity	LOD	LOQ	Solution Stability	Ruggedness
ID Tests (Specification Tests)										
GC, LC, TLC, CE	3	2	2	2	0	16	4	1	4	4
Related Substances Tests (Specification Tests)										
TLC	12	7	6	6	3	13	12	10	10	4
GC	9	8	7	9	4	9	8	8	9	6
HPLC	18	16	12	17	9	19	17	17	18	11
CE	2	2	2	2	1	2	2	2	2	2
Active Ingredient Assays (Specification Tests)										
GC, LC, TLC, CE	16	15	15	15	8	16	7	7	16	12
Preservatives and/or Anti-oxidants (Specification Tests)										
GC, LC, TLC, CE	18	17	13	18	6	14	6	6	18	10
Degradation Products (Stability Tests)										
TLC	12	6	6	7	13	14	11	11	11	5
GC	10	9	8	10	6	9	10	10	9	7
HPLC	17	14	13	17	9	18	16	16	17	10
CE	1	1	1	1	1	1	1	1	1	1
Active Ingredient Assays (Stability Tests)										
GC, LC, TLC, CE	15	16	12	16	9	16	7	6	16	11

(1) Repeatability (2) Reproducibility

Figure 5. Number of Responses Indicating that a Validation Parameter is Applied to a Test on the Finished Product at Various Stages in the Product Development Stage. Data is from a Survey of UK Pharmaceutical Companies by G.S. Clark.[49]

Ongoing validation activities may also be necessary during the routine utilization of an analytical procedure. System suitability determinations, frequently performed as a prerequisite to the generation of "real" data, represent essentially a validation at use. Re-validation of the analytical procedure may also be necessary as certain operational aspects of the method are changed during its routine and continuous application.

The focus of the remainder of this manuscript will be the formal or proper validation of an analytical method. The concepts of system suitability and re-validation are more completely addressed in the third part of this series.

VALIDATION PARAMETER GUIDELINES

Generally, two approaches can be utilized to determine which operational parameters should be included in a formal validation protocol. On one hand, one can look to the chemical literature to assess the practical state of the art among the practitioners of the desired methodology. In the case of chromatographic analyses, numerous reviews of implemented method validation strategies and procedures have been published. On the other hand, one can examine existing guidelines published by organizations with recognized authority within a given industrial arena (e.g., the FDA in the United States pharmaceutical industry). Both approaches are explored in this portion of the manuscript.

Trend Analysis

In 1990, T.D. Wilson, then a member of the Sterling Research Group, suggested that the question of how much and what kind of validation was necessary could be answered by examination of the pertinent literature, specifically published descriptions of liquid chromatographic procedures used in the analysis of drug substances and dosage forms. In a review published in 1990 which included 132 references,[28] Wilson summarized both the types of validation parameters typically reported in the pharmaceutical and analytical literature as well as the general validation approaches approaches employed. This information is further synthesized into Figure 3, which summarizes Wilson's results in the broadest general terms. For the purpose of this categorization, pharmaceutical products were divided into three general categories: drug substance, solid dosage forms; drug substance, solid and liquid dosage forms; and liquid dosage forms alone (including parenterals and aerosols). Thus for example, in 47 references related to the analysis of tablet

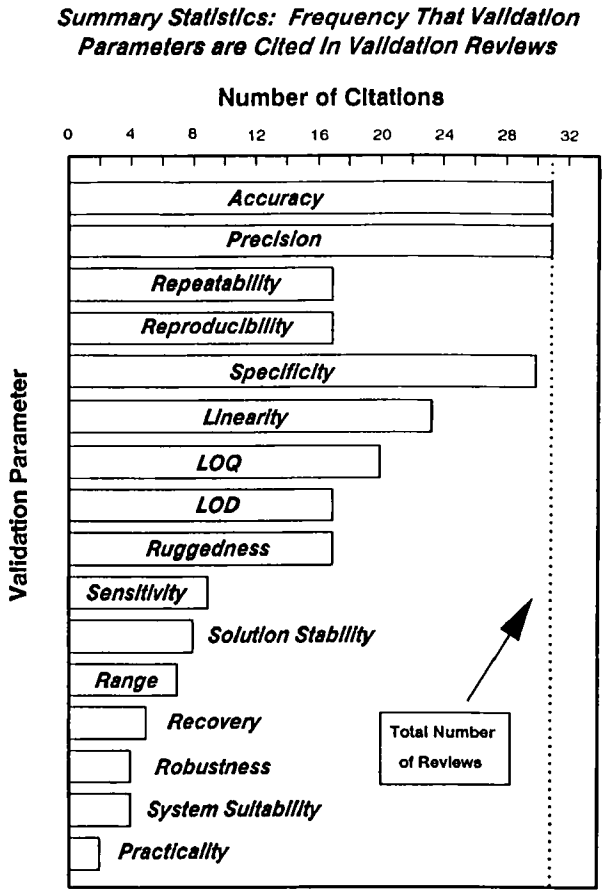

Figure 6. Summary Statistics, Frequency with which Specific Validation Parameters were referenced in General Manuscripts related to Analytical Method Validation.

and capsule dosage forms, accuracy was mentioned as a validation parameter in 40 instances, with 8 of the studies having performed the accuracy assessment by spiking at three different concentration levels.

C.S. Clarke, from Bristol-Myers Squibb, has recently published a survey of method validation procedures used in the testing of drug substances and finished products by many major research based pharmaceutical companies in the UK.[49] Portions of the results of the survey, summarized in Figures 4 and 5,

Data Elements Required for Assay Validation

(United States Pharmacopeial Convention)

Analytical Performance Parameter	*Assay Category I*	*Assay Category II*		*Assay Category III*
		Quantitative	*Limit Tests*	
Accuracy	Yes	Yes	(*)	(*)
Precision	Yes	Yes	No	Yes
Specificity	Yes	Yes	Yes	(*)
Limit of Detection	No	No	Yes	(*)
Limit of Quantitation	No	Yes	No	(*)
Linearity	Yes	Yes	No	(*)
Range	Yes	Yes	(*)	(*)
Ruggedness	Yes	Yes	Yes	Yes

(*) May be required, depending on the nature of the specific test.

Figure 7. Data Elements Required for Assay Validation per the USP. Table 2 from reference 2.

indicate which validation parameters were applied to particular tests at several stages in the product development cycle.

During the course of the literature review performed for this manuscript, 31 references, representing a cross section of authors from government, industry and academia, which specifically considered the issue of method validation from the perspective of which validation parameters were necessary were examined. Figure 6 represents a frequency distribution of the specific validation parameters which were mentioned in these manuscripts. For example, method accuracy was recognized as a universally necessary validation parameter in all 31 references. Validation parameters such as sensitivity were less universally mentioned but have a particular importance in specific applications.

Existing Guidelines

Several governmental bodies have published recommended general vali-

Important Validation Characteristics For Various Assay Types

(International Conference on Harmonisation: Draft Guidelines on ValidationProcedures for Pharmaceuticals; Availability)

Type of Assay / Requirement	Identification	Impurity Test		Content or Potency
		Quantitative	Limit	
Accuracy	-	+	-	+
Precision:				
Repeatability	-	+	-	+
Intermediate	-	+ (3)	-	+ (3)
Reproducibility	-	- (1)	-	- (1)
Specificity	+	+	+	+ (2)
Detection Limit	-	+	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- = not normally evaluated + = normally evaluated

(1) may be needed in some cases (2) may not be needed in some cases

(3) if reproducibility has been performed, intermediate is not needed.

Figure 8. Important Validation Characteristics for Various Assay Types per the International Conference on Harmonization (ICH), reference 8.

dation guidelines. For example, Figure 7 summarizes current validation guidelines established by the United States Pharmacopoeial Convention (USP).[2] The guidelines describe which validation procedures are necessary for compendial methods that fall under three general assay categories. Assay category I includes methods used for the quanititation of major components of bulk drugs or active ingredients (including preservatives). For these types of assays, in which the analyte should be present in relative abundance, parameters such as accuracy and precision are deemed necessary while measures of assay sensitivity (e.g., detection and quantitation limits) are not required. However, for assay category II, those used for impurities in bulk drugs and degradation products in finished product, the possibility that the quantity of analyte may be relatively small increases the importance of sensitivity-type validation parameters. Interesting differences in validation requirements arise in this category depending upon whether the assay is used to quantitate or only as a limit test.

Assay category III represents methods used to measure product perform-

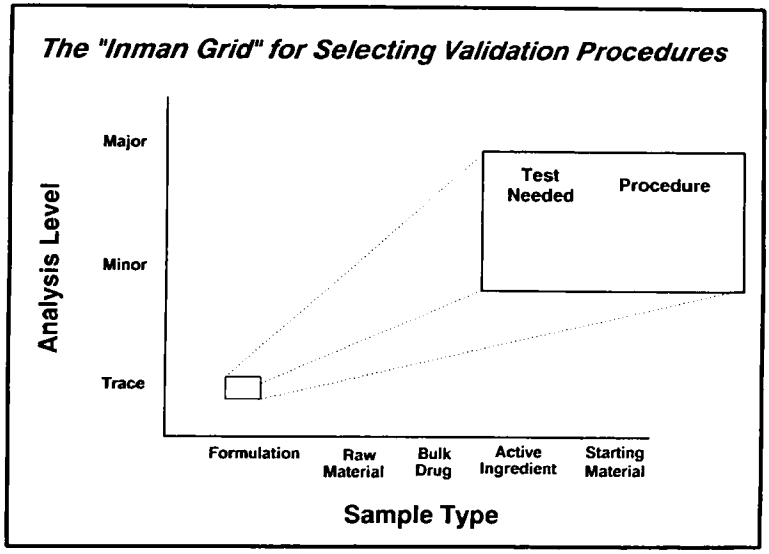

Figure 9. The "Inman" Grid for Selecting Validation Procedures. The assay is defined in terms of analyte level and matrix type and the validation parameters and general procedures are indicated.

perform-ance characteristics such as dissolution and/or release rate. Since this category includes any number of methods for any number of product properties, the general validation requirements are vague.

In a similar vein, the International Conference on Harmonization (ICH) has recently published its own list of important validation characteristics for various assay types[1] which is reproduced in Figure 8. In this classification, assays are categorized with respect to the utilization of the resulting data; e.g., identification [intended to ensure the identity of the analyte in the sample], impurity testing [intended to reflect the purity characteristics of a sample] and content/potency [intended to measure the analyte (active ingredient or major component)] present in a given sample.

In general the classifications of the USP and ICH are consistent, with the USP including ruggedness as a validation parameter due to the potential repeated use of compendial methods at numerous analytical sites.

General method validation guidelines were published by E.L. Inman and

Example of the "Inman Grid"

Validation Parameters; Major Component (>10%)

Sample Type	*Parameter*	*Validation Procedure*
Formulation	Linearity	Wide Conc. Range (10 to 200%)
		Narrow Conc. Range (50 - 150%)
		Both
	Precision	Multiple labs, instrument, analyst
	Recovery	Multiple spikes, multiple levels
	Specificity	Analyte-related substances
	Stability	Following day
	Matrix Effect	Wide Conc. Range (10 to 200%)
		Narrow Conc. Range (50 - 150%)
		Both

Figure 10. Portion of the Inman Validation Table for Major Components (>10%) in Formulation Samples. Based on the analyte level and sample matrix, the Table defines what validation parameters are necessary and suggests appropriate general validation procedures. From reference 19.

associates at Eli Lilly and Company in 1987.[19] The general outline of this manuscript (Figure 9) was to classify assays on the basis of analyte level and sample type. For each position along a two dimensional grid defined by these assay characteristics, specific validation procedures were defined. Thus, for example, for the validation of analytical methods used to quantitate major components in pharmaceutical formulations, these researchers suggest that linearity, precision, accuracy (recovery and matrix effect), specificity and stability are appropriate validation parameters (Figure 10). More specifically, they suggest that the precision determination, for example, would include a consideration of multiple labs, instruments and analysts, with the test for variation including a single run on potentially multiple product lots consisting of a minimum of ten replicates.

CONCLUDING REMARKS

It is clear from a review of the analytical literature that specific and unambiguous validation guidelines on even as general a topic as which validation parameters are appropriate for every specific analytical situation which might be encountered in the industrial and academic environment do not

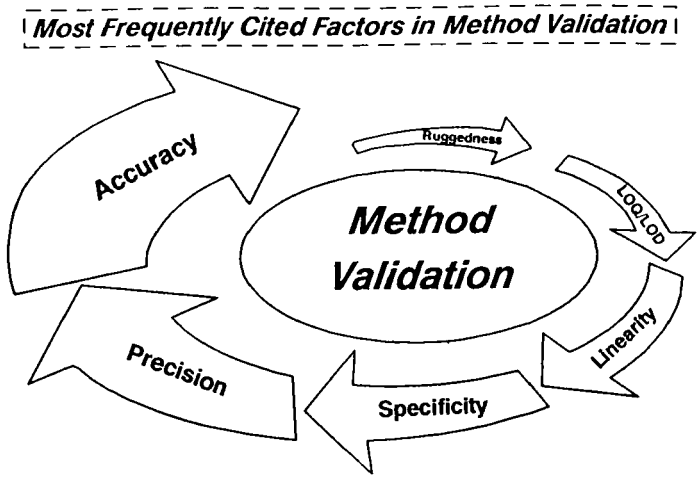

Figure 11. The Most Frequently Cited Validation Parameters in the papers reviewed for this manuscript. The most frequently cited validation parameters (accuracy and precision) would normally be a part of virtually every validation protocol while the use of the less frequently cited parameters would be application specific.

exist. The Holy Grail of one reference applicable for all situations has not surfaced and in a practical sense is impossible to envision. However, analytical professionals can receive meaningful guidance with respect to establishing appropriate validation parameters in particular situations from the research experiences and proposals published by their colleagues. Thus rather than relying on single reference which rigorously establishes invariant outlines of the validation study, the researcher has information which allows one to establish the boundaries of the validation study in a way that is meaningful for a specific analytical situation.

As illustrated in Figure 11, the literature clearly establishes that certain validation parameters (e.g., accuracy and precision) are applicable in virtually every analytical situation. Exclusion of such parameters from a validation protocol would most certainly require an extensive and scientifically rigorous justification.

Other parameters, such as specificity and linearity, are less universally applicable and thus their exclusion from general validation protocols could be somewhat less controversial. However, the need to include even these less common validation parameters in validation protocols under specific analytical

situations is clearly established in the literature.

This discussion represents a rather superficial examination of the analytical literature which considers the topic of primary cheomatogaphic method validation which can provide the validation specialist with potentially useful validation recommendations. The reader is strongly encouraged to examine applicable primary references in greater detail than can be presented herein.

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