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The validation of analytical methods for drug substances and drug products in UK pharmaceutical laboratories*

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Abstract: Results of a survey on method validation of analytical procedures used in the testing of drug substances and finished products, of most major research based pharmaceutical companies with laboratories in the UK, are presented. The results indicate that although method validation shows an essential similarity in different laboratories (in particular, chromatographic assay methods are validated in a similar manner in most laboratories), there is much diversity in the detailed application of validation parameters. Testing procedures for drug substances are broadly similar to finished products. Many laboratories validate methods at clinical trial stage to the same extent and detail as at the marketing authorization application (MAA)/new drug application (NDA) submission stage, however, only a small minority of laboratories apply the same criteria to methodology at pre-clinical trial stage. Extensive details of method validation parameters are included in the summary tables of this survey, together with details of the median response given for the validation of the most extensively applied methods. These median response details could be useful in suggesting a harmonized approach to method validation as applied by UK pharmaceutical laboratories. These guidelines would extend beyond the recommendations made to date by regulatory authorities and pharmacopoeias in that minimum requirements for each method validation parameter, e.g. number of replicates, range and tolerance, could be harmonized, both between laboratories and also in Product Licence submissions.

Keywords: *Method validation; drug substance; pharmaceutical products.*

Introduction

The analysis of drug substances and finished products within the pharmaceutical industry is carried out to satisfy both manufacturer and regulatory authority alike, about the quality, integrity and stability of the medicine administered to the patient. Several reports are available containing guidelines and recommendations for the validation of such analytical methods, which give an essentially similar list of parameters to be applied to the validation of a method. Some reports contain limited or no information concerning the detail of the validation parameters [1–4], whereas more recently published guidelines [5, 6] prescribe practical minimum requirements for each test.

The Pharmaceutical Analytical Sciences Group (PASG), which is an association of analytical chemists within the research based UK pharmaceutical industry, agreed to determine the current practice employed by its membership, and a questionnaire was completed by 20 laboratories, representing the major UK centres of pharmaceutical research and development, in 1992. The survey was subsectioned into method validation parameters employed for drug substance and drug products. Details were sought for different analytical methods, and also for each validation parameter. The results of this survey are contained in this report.

Results of Survey

The first part of the survey was intended to determine which validation parameters (e.g. accuracy, precision, etc) were applied to particular tests at the marketing authorization application (MAA) or new drug application (NDA) stage. This was further subdivided into bulk active or synthetic intermediate stage (Table 1) and finished product (Table 2). These tables indicate the number of companies who validate a particular test by application of the validation parameter. A response of "NA" indicates that the test is not performed for this material. A response of "No" indicates that no validation of this test is performed, although this does not indicate that the equipment used

*Note: This paper is presented on behalf of the Pharmaceutical Analytical Sciences Group (PASG).

Table 1
Number of responses indicating that the validation parameter is applied for a particular test on bulk active/synthesis material at the MAA/NDA stage (maximum response is 20 companies)

	NA	No	Accuracy	Precision (repeat)	Precision (reproduce)	Linearity	Sensitivity	Selectivity	LOD	LOQ	Soln. Stability	Ruggedness	Other
Specification tets													
ID tests													
Spectroscopic	10	3	2					7	3		1	2	1†
Chemical								1					
Chromatographic								5	1		1	1	
Solubility								1	1				
Physico-chemical													
Melting point	1	15	1	1				1					
Boiling point	9	9											
Light absorption	3	5		3	1	2		4	1	1	5	1	
Optical rotation	1	9	3	4	1	2		1			3		
Particle size	3	8	1	6	6	1	1	2	1	1	3	4	
DSC		2	1	1									
TGA		1	1	1									
Tests for impurities													
Sulphated ash		18						1	1				
Heavy metals		12	6	3		1		2	4			2	
Cation/anions													
— Wet	3	6	6	9	4	2	2	6	6	2	1	2	
— ISE	10	3	5	4	2	4	3	2	4	1	1		
— AA	2	4	13	12	5	9	5	8	10	8	4	4	

Related substances													
TLC	1		14	7	9	9	3	16	16	11	10	9	
GC	2		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		1	1	1	1	1	1		
Water content													
LOD	1	8	5	6	4	2	1	1	1	1	1	4	
KF		8	5	9	7	4	2	2	1	1	1	5	
Residual solvents		1	16	16	12	16	7	17	16	16	10	11	
Assays													
Titration	3	1	12	14	10	9	5	7	3	3	7	5	
Light absorption	6		12	12	9	13	7	9	4	3	10	6	
Chromatographic			18	17	15	18	10	17	8	8	16	14	
Stability tests													
Degradation products													
TLC	2		14	8	8	9	3	14	16	15	11	8	
GC	3		14	11	6	14	6	11	12	11	11	8	1*
HPLC			16	15	12	16	9	17	17	15	16	12	
CE			1	1	1	1	1	1	1	1	1	1	

*1 Company validates for carry-over.
†1 Company investigates polymorphism.
A 'NA' response indicates that this test is not performed within the company.
A 'No' response indicates that the test is performed but no validation is carried out.

Table 2
Number of company responses indicating that the validation parameter is applied for a particular test on finished product at the MAA/NDA stage (maximum response is 20 companies)

	NA	No	Accuracy	Precision (repeat)	Precision (reproduce)	Linearity	Sensitivity	Selectivity	LOD	LOQ	Soln. Stability	Ruggedness	Other
Specification tests													
ID tests													
Spectroscopic	3	6	2	2	1	1		9	3	1	1	2	1*
Chromatographic			3	2	2	2		16	4	1	4	4	
Pharmaceutical performance tests													
Tab. hardness	4	14											
Disintegration	1	18											
Dissolution													
— IR		2	13	11	10	16	5	10	2	3	13	12	
— ER	3		12	12	10	16	5	10	2	4	13	12	
Related substances													
TLC	4		12	7	6	6	3	13	12	10	10	4	
GC	8	1	9	8	7	9	4	9	8	8	9	6	
HPLC			18	16	12	17	9	19	17	17	18	11	1*
CE			2	2	2	2	1	2	2	2	2	2	
FIA			1	1	1	1		1	1	1	1	1	
Preservative/antioxidant assays													
Chromatographic	1		18	17	13	18	6	14	6	6	18	10	1*
Titration			4	4	3	3	1	3	1	2	4	2	

Spectrophotometric			3	3	1	3		1			3		
Assays for actives													
Light absorption	3		16	14	12	16	6	13	4	4	15	10	
Chromatographic			16	15	15	15	8	16	7	7	16	12	1*
Titration			2	1	2	2	2	2	1	1	2	2	
Stability tests													
Disintegration	2	16											
Dissolution — IR		2	13	10	10	16	5	12	2	3	14	11	
— ER	3		12	11	10	16	5	13	2	4	14	11	
Degradation products													
TLC	3		12	6	6	7	3	14	11	11	11	5	
GC	8		10	9	8	10	6	9	10	10	9	7	
HPLC			17	14	13	17	9	18	16	16	17	10	1†
CE			1	1	1	1	1	1	1	1	1	1	
Preservatives/anti-oxidant assays													
Chromatographic	1		18	17	15	18	7	14	7	7	18	11	1†
Titration			3	3	3	3	1	2	1	2	3	2	
Spectrophotometric			2	2	1	2					2		
Active assays													
Chromatographic			15	16	12	16	9	16	7	6	16	11	1*
Titration				1	1	1	1	1	1	1	1	1	

*1 Company validates for carry-over.
†1 Company validates for extraction times.
A 'NA' response indicates that this test is not performed within the company.
A 'No' response indicates that the test is performed but no validation is carried out.

is not calibrated. The likelihood is that for such tests, the most appropriate form of validation is the instrument calibration routine itself. For the purpose of this survey the term "ruggedness" has been used as defined in the USP XXII [2], and is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, e.g. different laboratories, different analysts, etc.

The highest degree of consistency is seen in the application of validation parameters to chromatographic techniques. This is particularly evident when considering HPLC where there is good agreement over which parameters should be applied to method validation. This reflects the universal application and dependence on the technique within the pharmaceutical industry.

Another area of consensus of validation parameters is the dissolution testing of finished products. More than half of the companies responded positively to the same seven validation parameters indicating a common understanding of the requirements for a valid dissolution procedure.

Comparison of method validation at the MAA stage, clinical trial stage and pre-clinical trial stage

The survey sought to determine how method validation at the MAA stage differed from that at the clinical trial and also the pre-clinical trial stage. Information about both bulk actives/synthetic intermediates and also finished products was determined. Table 3 summarizes the number of companies who responded that methods are similarly validated irrespective of the stage in the development of the drug substance or product.

(a) Drug substance. For the drug substance, a significant comment was that full validation should only be performed after the establishment of the final synthetic route, and that full validation should only be performed on assays of critical intermediates. Most companies who reported a difference in method validation at the pre-MAA stage indicated that a limited validation was performed, e.g. limited precision or selectivity data. Other companies only performed full validation of the assay procedure and did not validate intermediate assays.

(b) Finished product. Similar comments (to the drug substance) were made about the validation of methodology for finished products at the pre-MAA stage to those given for the drug substance. The greater majority of companies fully validate methods at the clinical trial stage, however, only four companies fully validate methodology at the pre-clinical trial stage. Pre-MAA method validation typically omits ruggedness and selectivity parameters and includes only limited precision testing. One company reported that at the pre-MAA stage only the finished product assay was fully validated.

Detail of validation parameters

The survey sought to determine the specific details of how each validation parameter was applied to a particular sample type, i.e. bulk drug/finished product and also the test, e.g. assay, related impurities, residual solvents, etc. The following validation parameters were included in the questionnaire: accuracy, precision (repeatability), precision (reproducibility), linearity, limit of detection/quantitation, ruggedness/robustness, selectivity and system suitability.

For many of the the validation parameters, the detailed response from each of the companies revealed there was much diversity in how each parameter is performed, e.g. for determining the linearity of the assay for finished products, the following concentration ranges are used. Six companies indicated that they validate linearity over the 50–150%

Table 3
Comparison of the method validation protocol applied at MAA/NDA stage to the development stage (i.e. clinical trial or pre-clinical trial stage). The number of responses indicate the number of companies who do not differentiate between the development stage and MAA/NDA stage with respect to method validation

	Number of responses	
Development stage	Drug substance/Synthetic intermediate	Finished product
Clinical trial	11	12
Pre-clinical trial	4	4

range: at all other ranges the response was only returned by a single company.

Concentration range used by different companies for finished product linearity determination

0–120%
0–125%
0–150%
0–200%
10–125%
10–150%
10–200%
20–150%
20–200%
25–150%
40–140%
50–150%
70–130%
75–125%

In order to obtain a "typical" profile of how the validation parameters are applied to each test method, the median response has been extracted from the questionnaire data, and is presented herein.

(a) Bulk drug assay and impurity assay (chromatographic). The median response for the detail of the validation parameters for bulk drug chromatographic assay and impurity assay are shown in Tables 4 and 5, respectively. The same number of replicate analyses or samples in the linearity determination are applied to both the drug substance and the impurity assay. Wider tolerance is generally accepted for the accuracy and precision method validation parameters for an impurity assay, for which the limits of detection and quantitation are also determined (3 × S:N and 10 × S:N ratio, respectively). The linearity is

Table 4
Bulk drug assay (chromatographic) median response

	Number of samples	Range	Tolerance
Accuracy	6	50–150%	±2%
Precision (repeatability)	6		±2%
Precision (reproducibility)	6		±2%
Linearity	6	20–150%	r^2 >0.999 95% CI of intercept includes zero
LOD	N/A		
LOQ	N/A		

Ruggedness — Parameter evaluation, e.g. Mobile phase, pH, Columns, etc.
— Different days, analysts, labs, equipment
Selectivity — Interference from critical components
System suitability

Majority response	Minority response
Precision	Accuracy
Selectivity (resolution)	Linearity
Chromatographic (e.g. k')	S:N ratio
	Selectivity (peak homogeneity)
	Stability of solution
	LOD

N/A = Not applied.
CI = Confidence interval.

Table 5
Bulk drug impurity assay (chromatographic) median response

	Number of samples	Range*	Tolerance
Accuracy	5	50–150%	±20%
Precision (repeatability)	6		±2%
Precision (reproducibility)	6		±5%
Linearity	6	20–150%	r^2 >0.99 95% CI of intercept includes zero
LOD	3× S:N		
LOQ	10× S:N		

Ruggedness, selectivity and system suitability are as in Table 4.
*Per cent of upper specification limit.

most commonly determined by the correlation coefficient and the intercept is determined as not significant from zero, if the origin is included in the 95% confidence interval.

(b) Finished product assay and degradant assay (chromatographic). The median response for the detail of the validation parameters for the finished product assay and degradant assay are shown in Tables 6 and 7, respectively. Essentially the same number of replicate analyses or samples as used in the linearity determination are applied to both the finished product assay and the degradant assay. A wider tolerance is accepted for the degradant assay (±10%) than with the potency assay (±2%). The concentration range for which the linearity is determined for degradants is 0–2% of the finished product potency label. The limits of detection and quantitation are applied to the degradant assay procedure and are determined as 3 × S:N and 10 × S:N ratio, respectively. Linearity is similarly determined as for the bulk drug, i.e. the correlation coefficient and the 95% confidence interval at the intercept.

(c) Residual solvents (bulk drug). The details of the validation parameters applied to the determination of residual solvents in bulk drug samples are given in Table 8. The accuracy is determined over 50–150% of the upper specification limit. The linearity is usually determined over the 10–200% range, and the limit of detection (LOD) and limit of quantitation (LOQ) are determined often from the S:N ratio, but also by sequential dilution and

Table 6
Finished product assay (chromatographic) median response

	Number of samples	Range*	Tolerance
Accuracy	6	75–125%	±2%
Precision (repeatability)	6		±2%
Precision (reproducibility)	6		±5%
Linearity	6	25–150%	r^2 >0.999 95% CI of intercept includes zero
LOD	N/A		
LOQ	N/A		

Ruggedness, selectivity and system suitability are as in Table 4.
N/A = Not applied.
* Per cent of label claim.

Table 7
Finished product degradant assay (chromatographic) median response

	Number of samples	Range	Tolerance
Accuracy	6	50–150%*	±10%
Precision (repeatability)	6		±2%
Precision (reproducibility)	5		±3%
Linearity	6	0–2%†	95% CI of intercept includes zero
LOD	3× S:N		
LOQ	10× S:N		

Ruggedness, selectivity and system suitability are as in Table 4.
* Per cent of upper specification limit.
† Per cent of active drug component in the formation.

Table 8
Residual solvents bulk drug median response

	Number of samples	Range*	Tolerance
Accuracy	6	50–150%	±20%
Precision (repeatability)	6		±5%
Precision (reproducibility)	5		±5%
Linearity	5	10–200%	r^2 >0.99 95% CI of intercept includes zero

LOD, LOQ are determined for residual solvents.
* Per cent of upper specification limit.

determination of an observable chromatographic peak.

(d) Dissolution testing. The details of the validation parameters applied to dissolution methodology are given in Table 9. The same number of samples are generally used for accuracy, precision and linearity as are used in the bulk drug and finished product assays. However, the validated accuracy and linearity ranges are broader, reflecting the nature of dissolution samples which may contain drug concentrations over a range from zero, to complete release of the drug substance from the formulation. Selectivity validation, i.e. non-interference from impurities and excipients was noted as a particularly important validation requirement, as the analytical endpoint of dissolution samples is often a non-specific UV determination. The need for determining the stability of dissolution solutions is almost universally accepted.

(e) System suitability testing. The survey investigated the application of system suitability testing procedures. The responses indicated how validation parameters were applied to chromatographic procedures, and a summary of these responses is given in Table 10. A "typical" system suitability test applied to a chromatographic method would include tests for precision (replicate injections of a standard solution), selectivity (ability of the system to discern critical components) and chromatographic performance (e.g. k', tailing factor and column efficiency). The application of these system suitability procedures has been described as a "traditional approach" [7] and even though the use of peak tailing factors and column efficiency has been questioned, they presently appear to be extensively used in the UK pharmaceutical industry. The current USP Pharmacopeia [2] describes chromatographic system suitability tests for the precision, selectivity and tailing factor as being useful but does not preclude the use of other operating criteria. Other such system suitability criteria are applied by different companies, and are indicated in Table 4 as a minority response.

(f) Precision testing of multiple strength formulations. When several strength products are available, which may be manufactured for example, by the compression of different weights from a common blend, different approaches are taken for testing of the assay. Table 11 shows that for such product strengths the trend is to precision test only the maximum

Table 9
Dissolution testing (finished product) median response

	Number of samples	Range*	Tolerance
Accuracy	6	40–120%	±2%
Precision (repeatability)	6		±2%
Linearity	6		
		25–150%	
Selectivity:	Interference from impurities/excipients		
Solution stability:	Performed		

*Per cent of the theoretical 100% dissolution concentration.

Table 10
System suitability testing

Validation parameter	Company responses Yes:No
Accuracy	4:16
Precision	14:4
Linearity	3:15
LOD	9:11
S:N ratio	1:19
Selectivity — resolution	20:0
— peak homogeneity	1:19
Stability of analytical solutions	1:19
Chromatographic parameters e.g. k', tailing factor, column efficiency	11:9

Table 11
Summary of precision validation of multiple strength formulations

	Every strength	Maximum and minimum strength only
Precision (repeatability)		
Yes	8	12
No	8	5
Precision (reproducibility)		
Yes	5	10
No	7	3

and minimum assay strengths. This statistic however, may hide the fact that it is less common to manufacture more than two strengths of the product. If there are several strength products, and the potency range is large, intermediate formulations to the maximum and minimum strength may be precision tested. There are however, a considerable number of companies who will perform precision testing on every strength of formulated product.

Conclusions

It is clear from this survey, that within the UK Pharmaceutical industry, method validation approaches show an essential similarity although there is much diversity in the finer detail. The greatest degree of consistency appears to be in the validation parameters applied to chromatographic procedures. This is particularly the case for HPLC, which is a reflection of the universal application of the technique within the industry. There is, however, a diversity in the detail applied by individual companies to method validation parameters, e.g. number of repetitions, ranges and tolerance. This may be a reflection of the type of pharmaceutical dosage forms manufactured, and also the different submission requirements expected by national regulatory authorities.

In many cases, the median response values for the validation parameter details (Tables 4–10) is also the response of the majority of the companies surveyed. These values could represent useful proposals for a harmonized minimum requirement for method validation within the pharmaceutical industry. Method validation is performed to satisfy both the user and the regulator that the procedures used to test a product are both accurate and reliable. A harmonized guideline would be useful in giving direction to the originator of the validation data, and also the approver who should receive data in a format consistent from one submission to another. However, if a harmonized guideline were accepted for method validation, it would be inevitable that there would be examples where additional testing would be necessary. This survey has been useful in assessing the approaches to method validation in the UK pharmaceutical industry in 1992 but is limited in application in that it is a snapshot of current awareness which out of necessity is continually under review.

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