ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1427–1436

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Inspection by variables as an acceptance criterion in bioanalysis — a proposal

Krzysztof A. Selinger

Department of Clinical Pharmacology, Glaxo Research Institute, Five Moore Drive, Research Triangle Park, NC 27709, USA

Received for review 1 February 1995; revised manuscript received 15 May 1995

Abstract

Inspection by variables is proposed as an acceptance criterion for use in bioanalysis. The criteria currently used are deficient either by ignoring the issues of precision (fixed range) and/or accuracy (99% confidence interval), not being able to provide immediate answers (quality charts), or even not being scientifically justified (fixed range). Inspection (sampling) by a variables procedure was originally developed to drive the quality of military supplies (MIL-STD-414) and was consequently incorporated in ISO 3951 as a part of industrial quality control. It is based on the concept of acceptable quality level (AQL), which is the maximum per cent defective (number of results outside of specification per 100 results) that can be considered satisfactory as a process average. It also correlates sample size with batch size. An AQL of 6.50% is proposed as a standard with specification set at ±20% or ±15%, which should result in coefficients of variation of approximately 15% and 12%, respectively. This concept has been applied post factum to 14 authentic data sets, thus proving its utility and validity.

Keywords: Acceptance criteria; Accuracy; Bioanalysis; Inspection by variables; Precision; Quality control

1. Introduction

The most common criterion used to accept bioanalytical data is the fixed range, which is endorsed by both the Conference Report [1] and the DIA Consensus Statement [2]. It requires that quality control samples (QCs) at three levels, including high, medium, and low, be analyzed in duplicate with each batch of clinical samples. Additionally, the back-calculated concentrations of four out of the six of these QCs, including one QC at each level, must be within 20% of nominal for acceptance of an analytical batch (4–6–20 rule). The precision, measured as coefficient of variation, should be no greater than 15% at all levels except the lower limit of quantitation (LLOQ), which is 20%.

In industrial practice, stricter norms are frequently used. Very commonly acceptance criteria are set at 10, 15, and 20% (from the highest QC concentration to the lowest), or 10, 10, and 15%, or a similar combination. Secondary acceptance rules may also be used. For example deviations of standards from the nominal concentration are set at 15% except for the LLOQ, where it can be 20%; the number of calibration standards that can be rejected from regression is limited (too great a number of rejected standards suggest a lack of robustness and analytical problems); a correlation coefficient of at least 0.99 is required; and drug-free samples must be free of interferences. Less common are some other rules, such as an even distribution of positive and negative bias, the rejection of values between unacceptable QCs, the inclusion of assay-specific secondary quality controls, etc.

SSDI 0731-7085(95)01590-6

In the opinion of this author and others, this element of the Conference Report invites criticism and needs improvement. Karnes and March [3] presented alternatives to the fixed range: a 99% confidence interval, Westgard multirules, range charts combined with the two-thirds of run rule (four out of six QCs) and the bracketed approaches. Lang and Bolton [4], even before the Washington Conference, proposed to use control charts for the acceptance of quality control data. Hartmann et al. [5] calculated that in order to obtain mean values within the limits of ±15% with a probability of 95%, the bias and coefficient of variation (CV) should be 8% or lower with $n = 5$. This agrees with the data of Causey et al. [6], who showed that the two-thirds of run approach can be justified scientifically only if the rejection criterion is ±10%, but not ±20%. The questionable scientific and statistic validity of the 4–6–20 rule has been shown by Kringle [7]. The fixed range approach accepted by the Conference is mostly pragmatic, it describes quite well the current state of bioanalysis that authors of the report and the industry at large were comfortable with. The fixed range criteria approach confuses precision and accuracy, applies the same rules to all assays disregarding batch sizes and the needs of a project, and is not based on statistics in any strict sense.

A good set of acceptance criteria should be scientifically valid, should be able to detect errors (improve quality of data) and false alarms (improve productivity), should be easy to use, and should provide immediate answers. The Conference Report recognized that a confidence-interval approach is an acceptable alternative for acceptance criteria.

In the opinion of this author, assaying biomedical samples is no different from manufacturing a widget of specified parameters. Hence, bioanalysis should reach to the wealth of research and literature on quality control in the manufacturing industries [8,9]. One of these concepts, inspection by variables, also referred to as acceptance sampling by variables, seems to be very attractive and possible to adapt easily to bioanalysis [8–10]. The goal of this paper is to apply inspection by variables to bioanalysis in such a way as to accept the customs and established ways of the industry, to show its advantages, and to present it as a scientifically valid alternative to the fixed approach. To the best knowledge of the author, no one has yet tried this approach.

2. Inspection by variables

Sampling systems were developed for military contracts during World War II and are currently accepted as British, American, and International Standards, most notably as MIL-STD-414 [11] and ISO 3951 [12] for inspection by variables. The sampling plans for these standards are based on three strategies: (1) sample size is related to the batch size; (2) an acceptable quality level (AQL) divides the risks between the producer and the consumer equitably; and (3) rules are switched from normal to tightened which permits quality to be driven in the interest of the consumer.

A short explanation of the terms is needed. The producer's (bioanalyst's) risk, α, is the probability that a "good" batch will be rejected by the sampling plan. This risk varies from 0.01 to 0.10, and frequently is fixed at 0.05. The consumer's (e.g. pharmacokinetist's, or regulatory authorities') risk, β, is the probability that a "bad" batch will be accepted. The term "good" or "bad" means that it is within or outside of specifications, respectively. AQL is the maximum per cent defective (number of results outside of specification per 100 results) that can be considered satisfactory as a process average. AQL cannot be set by scientific reasoning, it is either set by the bargaining between the producer and the consumer, or it is arbitrarily chosen. AQL can be established on the basis of comparing the cost of inspection to the costs of accepting a substandard product. The sampling plans can utilize the standard deviation, s, or the standard deviation of the population, σ, if known, or the range, R. There are plans for single, double, or combined specification limits. Inspections may be normal, tightened, or reduced. Readers interested in the details are advised to study books by Juran [8] and/or Duncan [9].

2.1. Inspection by variables applied to bioanalysis

The procedures described in MIL-STD-414 and ISO 3951 are very similar, but not identical. The main differences (pertinent to this limited application of inspection by variables) are that MIL-STD-414 offers two procedures known as Form 1 and 2, while ISO 3951 accepts only Form 1 as the sole procedure. Also the military standard provides tabulated parameters for AQL up to 15% while ISO 3951

Table 1
Data set used in the calculations

Statistical parameters	Nominal	A		B	
		Assayed	%	Assayed	%
	3.00	3.27	109.00	3.55	118.33
	3.00	2.90	96.67	2.41	85.55
	50.00	47.56	95.12	56.20	112.40
	50.00	52.59	105.18	57.11	114.22
	90.00	94.31	104.79	98.90	109.88
	90.00	84.92	94.36	101.24	112.49
Mean			100.852		108.812
s			6.216		11.735
Qu			3.080		0.953
Ql			3.355		2.455
k at 2.50 = 1.29			Accept		Reject
k at 4.00 = 1.11			Accept		Reject
k at 6.50 = 0.916			Accept		Accept
k at 10.00 = 0.717			Accept		Accept
k at 15.00 = 0.497			Accept		Accept
MSD at 2.50 = 13.28			Accept		Accept

only up to 10%. The sampling plan presented below uses $\alpha = 5\%$, (95% probability that a batch meeting specification criteria will be accepted), and $\beta = 10\%$ (10% probability that a substandard batch will be accepted anyway). Here we limit our considerations to the standard deviation s, although one may consider σ known based on validation results. For AQL, our considerations will be limited to AQL 2.50–15.00%, where most of the bioanalytical data seem to fall, as was discovered after a quick scanning of some authentic data. ISO 3951 does not support AQLs greater than 10%, although it provides operating characteristic curves (OC) for 15%.

Let us try to use this for the acceptance or rejection of the hypothetical data shown in Table 1; where column A presents very accurate and precise data, and column B presents a much worse set of data. The specification limits are set here at $\pm 20\%$, and only normal inspection is considered.

(1) Upon completion of an analytical run, calculate the accuracy of the QCs and express it in per cent according to the following formula

$$\text{Accuracy}(\%) = \frac{\text{Assayed concentration}}{\text{Nominal concentration}} \times 100\%$$

One has an option to use the observed mean instead of the nominal concentration, as an assay bias is rarely zero. Such an observed mean should be established after assaying a number of QCs ($n \geq 6$) from the same batch.

(2) Calculate the mean $\bar{x}$ and the standard deviation s for all QCs in this batch.

(3) Review the data for outliers. Do not include in the calculations any results disqualified for valid analytical reasons, such as bad chromatography or errors in processing. Carry out a simple outlier test like Dixon's. Do not include the outliers in the calculations, if present.

(4) From Table 2 select a code letter according to the batch size and general inspection level. Generally, inspection level II is used, although level I here is recommended. The number of clinical samples (plus QCs, but not including calibration standards and blanks) in a typical batch is 51–90, hence letter D is

Table 2
Code letters and inspection levels (excerpts from Table I-A, ISO 3951-1989)

Lot or batch size	General inspection levels		
	I	II	III
2–8			C
9–15		B	D
16–25	B	C	E
26–50	C	D	F
51–90	D	E	G
91–150	E	F	H
151–280	F	G	I
281–500	G	H [a]	J
501–1200	H	J	K

[a] Use H for lot size 281–400, and I for lot size 401–500.

Table 3 [a]
Single sampling plans for normal inspection (master table): "*s*" *method*

Sample size letter code	Sample [b] size	Acceptable quality level (normal inspection) [c]											
		0.1	0.15	0.25	0.40	0.65	1.00	1.50	2.50	4.00	6.50	10.00	15.00 [d]
		k	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
B	3	–	–	–	–	–	–	–	1.12	0.958	0.765	0.566	0.341
C	4	–	–	–	–	–	1.45	1.34	1.17	1.01	0.814	0.617	0.393
D	5	–	–	–	–	1.65	1.53	1.40	1.24	1.07	0.874	0.675	0.455
[e]	6	–	–	–	n a [f]	n a [f]	1.57	1.45	1.29	1.11	0.916	0.717	0.497
E	7	–	–	2.00	1.88	1.75	1.62	1.50	1.33	1.15	0.955	0.755	0.536
F	10	–	2.24	2.11	1.98	1.84	1.72	1.58	1.41	1.23	1.03	0.828	0.611
G	15	2.42	2.32	2.20	2.06	1.91	1.79	1.65	1.47	1.30	1.09	0.886	0.664
H	20	2.47	2.36	2.24	2.11	1.96	1.82	1.69	1.51	1.33	1.12	0.917	0.695
I	25	2.50	2.40	2.26	2.14	1.98	1.85	1.72	1.53	1.35	1.14	0.936	0.712
J	35	2.54	2.45	2.31	2.18	2.03	1.89	1.76	1.57	1.39	1.18	0.969	0.745
K	50	2.60	2.50	2.35	2.22	2.08	1.93	1.80	1.61	1.42	1.21	1.00	0.774

[a] Table 3 is based on Table II-A ISO 3951-1989.
[b] The sample size is equal to the number of QCs per batch which is equal to *n*.
[c] All AQL values are in per cent defective.
[d] Values taken from MIL-STD-414.
[e] Not official; values are calculated by the author.

chosen. Note if the batch size is smaller than usual, either letter C or B can be selected.

(5) Determine the sample sizes and acceptance constant, *k*, for normal inspection using Table 3. The official tables do not contain *k* for $n = 6$; these have been calculated by the author. The generally accepted norm in bioanalysis is to have $n = 6$ QCs per batch. This corresponds well to size code D and sample size 5. The sixth QC may serve as an insurance against loss of a QC or the presence of outliers.

(6) Calculate the upper quality statistic, *Qu*, and the lower quality statistic, *Ql*, according to the formulae

$$Qu = (\text{upper specification limit} - \bar{x})/s$$

$$Ql = (\bar{x} - \text{lower specification limit})/s$$

The upper and lower specification limits are here 120% and 80%, respectively. The *Qu* and *Ql* are 3.080 and 3.355 for set A, and 0.953 and 2.455 for set B.

(7) From Table 3, find the quality constant *k* at the appropriate AQL and letter code D. If both the calculated *Qu* and *Ql* are greater than *k* from the table, the batch will be accepted at that AQL; if either *Qu* or *Ql* is smaller than *k*, the batch must be rejected (or accepted at a different AQL). Additionally, *s* must be smaller than the maximum standard deviation (MSD). MSD is calculated as

$$\text{MSD} = f(\text{Upper specification limit} - \text{Lower specification limit})$$

The values of *f* are presented in Table 4. Again, values of *f* are not given in these standards for $n = 6$, and were calculated by the author with an accuracy of $\pm 1\%$. It is easy to calculate that the MSDs at a ± 20 specification limit, $n = 6$ and AQL 2.50%, 4.00%, 6.50%, 10.00%, and 15.00% are 13.28, 14.28, 15.68, 17.36, and 19.92, respectively. The run is rejected at once if *Qu* or *Ql* is negative, which occurs if the mean is greater than 120%, or smaller than 80%.

Set A is acceptable at any AQL, while set B must be rejected at AQL 2.5 and 4% and may be accepted at AQL 6.5, 10 and 15%.

2.2. Application of inspection by variables to model data sets

One may ask how sensitive the sampling scheme is toward acceptance or rejection of typical analytical runs. Several model cases are presented in Table 5. The model cases were chosen to represent several scenarios, including a very good set of data with a CV of 7.7% (column A), sets of data that have an increasingly aberrant result (columns B–E), and sets of data with two increasingly aberrant results (columns F–J). The specification is set at 80–120% for each data set and the resulting AQL is observed.

Table 4
Values of f for maximum standard deviation (MSD); "s" method (excerpt from Table IV ISO 3951-1989)

Sample size	Acceptable quality level (normal inspection)											
	0.10	0.15	0.25	0.40	0.65	1.00	1.50	2.50	4.00	6.50	10.00	15.00
3	–	–	–	–	–	–	–	0.436	0.453	0.475	0.502	0.538
4	–	–	–	–	–	0.339	0.353	0.374	0.399	0.432	0.472	0.528
5	–	–	–	–	–	0.308	0.323	0.346	0.372	0.408	0.452	0.511
6 [a]	–	–	–	–	–	–	–	0.332	0.357	0.392	0.434	0.498
7	–	–	–	0.242	0.253	0.280	0.295	0.318	0.345	0.381	0.425	0.485
10	–	–	0.214	0.224	0.235	0.261	0.276	0.298	0.324	0.359	0.403	0.460
15	0.188	0.195	0.202	0.211	0.222	0.248	0.262	0.284	0.309	0.344	0.386	0.442
20	0.183	0.190	0.197	0.206	0.216	0.242	0.255	0.277	0.302	0.336	0.377	0.432
25	0.180	0.187	0.193	0.203	0.212	0.238	0.251	0.273	0.297	0.331	0.372	0.426
35	0.176	0.183	0.189	0.198	0.208	0.232	0.245	0.266	0.291	0.323	0.364	0.416
50	0.172	0.178	0.184	0.194	0.203	0.227	0.241	0.261	0.284	0.317	0.356	0.408

[a] Not official; values calculated by the author.

(1) Column A: data are accepted at AQL better than 2.50%, i.e. less than 2.5% of the results would be outside the 80–120% limits.

(2) Column B: one slightly aberrant result at 120% of nominal; results still acceptable at AQL 2.5%.

(3) Column C: the aberrant results at 125% of nominal, results still acceptable at AQL 2.5%.

(4) Column D: at an error of +30%, the AQL is 4%; Dixon's outlier test does not reject the aberrant result.

(5) Column E: the error grows to +35% and causes rejection of this data point as an outlier; the batch will be accepted at AQL better than 2.50% using k at $n = 5$. The same will happen with any other error greater than 35%.

The conclusion is that a single aberrant QC will not cause rejection of the whole batch.

(6) Column F: two results are at limits of specification and of opposite signs at +20% and −20%; the batch can be acceptable at AQL 4%.

(7) Column G: if the two errors grow by an additional 5%, the batch can be accepted at AQL 10%.

(8) Column H: two errors of the same sign at +20% are introduced; the batch is still acceptable at AQL 4%.

(9) Column I: if the two errors grow by an additional 5%, the batch can be accepted at AQL 10%.

(10) Column J: if the two errors grow still further, the batch reaches limits of acceptability — 15%. Dixon's outlier test will not identify any outliers.

The conclusion is that the procedure will not accept a batch with two large errors greater than 25–30% disregarding the sign of error. Hence, at least five QCs must be within ±20% for the run to be acceptable; and of course at least one of them will be the low QC, where the potential for errors is usually the greatest. If the results are very accurate, the relative imprecision of a CV of approximately 10% does not hurt the run. If the results are relatively inaccurate with a bias of approximately 15%, the precision must be much better for a run to be accepted. Inspection by variables will not accept batches that are both imprecise and inaccurate.

(11) Column K: a scenario is shown where three perfect QCs are present and the other three are outside of specification by a fraction of a per cent. The batch would be rejected by the traditional fixed range criteria, but it is still acceptable at AQL 6.5% according to the sampling by variables scheme.

(12) Column L: a scenario is shown where four barely acceptable QCs are present, while the other two are outside of specification by a large margin. The batch would be acceptable by the traditional fixed range criteria, but it is rejected using sampling by variables due to lack of precision.

Cases K and L are of particular importance to this author, who for a long time has looked for acceptance criteria which eliminate such irritating nonsense as accepting a run on the strength of four barely acceptable QCs, while rejecting good data for the missing three QCs by a fraction of a per cent.

3. What is an appropriate AQL?

So far, no one has tried to use the inspection by variables in bioanalysis, so the correct AQL is unknown. If one wants to use the Conference Report [1] as the specification (limits ±20%; CV is approximately equal to 15%), then the AQL should be set at 6.5%. In the proposed procedure s is approximately equal to the coefficient of variation (CV), and the MSD is the limiting factor. For a number of QCs, $n = 6$ at an AQL of 6.5, the MSD (approximately equal to the CV) is equal to 15.68 (see Table 4), which is close enough to the specification.

To verify these acceptance criteria, this author analyzed data from 14 completed randomly chosen bioanalytical projects. Specification was chosen as ±20%, normal inspection. These projects were carried out either internally in the Clinical Pharmacology Laboratory at the Glaxo Research Institute, or by leading contract organizations. The projects' results were already accepted, mostly by the fixed range acceptance criteria at 10, 15, and 20%. A description of these projects and results is presented in Table 6.

Out of these 14 projects, two (D and J) raise concern in that the general quality is lower than in the other projects, and in that some batches are rejected after all by inspection by variables. In project D, one formally acceptable batch was rejected by the proposed procedure; the case was similar to the scenario L above.

In project J, all five questionable runs (rejected entirely or acceptable at AQL above 6.50%) had one acceptable low QC at the limits of acceptability (mean 87.6%), and the other an unacceptable low QC (mean 68.2%). Upon close inspection it is evident that either the low QC was underspiked, or an inappropriate response model was selected, which resulted in underestimated concentrations at low concentrations. This mistake went unnoticed or ignored, resulting in a general low analytical quality of the project. The verdict delivered by the inspection by variables is harsh, but seems to be fair in this case.

Worth recommending is the use of stricter rules than those endorsed by the Conference, such as acceptance limits set at ±15%, which is closer to the very common 10, 15, 20% rule. In this case acceptable, an AQL could be even 15%, as the MSD (approximately the CV) at this level is 14.94. However, this would do little for the quality of projects, as the percentage of runs accepted at ±20%, AQL 6.50 is 97% as compared to 95% at ±15%, AQL 15.00. A recommendation is made here on the basis of a balance between productivity and quality to consider AQL at 6.50% as a standard or at least a starting point in bioanalysis. The price to pay for narrowing the specification range from 120–80% to 115–85% is approximately 11% in sample throughput.

4. Discussion

The author would prefer to use the inspection by variables exactly as it is recommended in ISO 3951, i.e. general inspection level II, which for a typical batch of 51–90 samples requires seven quality controls. However, the accepted practice in bioanalysis is to have six QCs. The proposed modification is aimed at making the inspection by variables more palatable for the bioanalytical community.

The use of inspection by variables as acceptance criterion has several advantages as compared to other systems of acceptance criteria. This is the only system that incorporates precision, accuracy, and agreement with specification, provides immediate answers, and is based on solid statistics. Other systems lack at least one or more of these elements. The fixed range is arbitrary, does not include precision, and uses the same criteria for all studies. The 99% confidence interval approach ignores accuracy and specification. The quality control chart system may not provide immediate answers, as even later batches may decide the acceptance or rejection of the first run analyzed.

A balance is needed between the quality and productivity. According to Caulcutt [13]: "The statistician has no desire to tell the chemist what constitutes an important change. The statistician would certainly not wish the chemist to equate statistical significance with practical importance. Only the chemist can decide what is important and he must take into account many factors including the possible consequences of his decision".

Inspection by variables as acceptance criteria in bioanalysis offers other advantages. It is based on ISO 3951, which has been accepted by dozens of technologically leading countries. It may permit us to tie the statistical power of a pharmacokinetic study with the analytical performance of a method, as the α and β may be selected by bargaining between the producer (analyst) and the consumer (pharmacokinetist).

Table 5
Model data sets for testing by inspection by variables

	Statistical parameter	A	B	C	D	E	F	G	H	I	J	K	L
	–	109.5	120.0	125.0	130.0	135.0[a]	120.0	125.0	120.0	125.0	130.0	120.1	119.8
	–	92.1	92.1	92.1	92.1	92.1	80.0	75.0	120.0	125.0	130.0	99.5	72.2
	–	107.0	107.0	107.0	107.0	107.0	107.0	107.0	107.0	107.0	107.0	79.8	82.7
	–	92.9	92.9	92.9	92.9	92.9	92.9	92.9	92.9	92.9	92.9	101.1	125.1
	–	105.5	105.5	105.5	105.5	105.5	105.5	105.5	105.5	105.5	105.5	120.2	109.9
	–	95.8	95.8	95.8	95.8	95.8	95.8	95.8	95.8	95.8	95.8	98.7	92.3
	Mean	100.467	102.217	103.050	103.883	98.660	100.200	100.200	106.867	108.533	110.200	103.233	100.333
	s	7.729	10.772	12.481	14.277	7.084	13.763	16.716	11.527	13.859	16.267	15.235	21.217
Specification	*Qu*	2.527	1.651	1.358	1.129	3.012	1.439	1.185	1.139	0.827	0.602	1.101	0.927
limit	AQL	<2.50	<2.50	<2.50	<4.00	<2.50	<2.50	4.00	4.00	10.00	15.00	6.50	6.50
120–80%	*Ql*	2.648	2.062	1.847	1.673	2.634	1.468	1.208	2.331	2.059	1.857	1.525	0.958
	MSD	<2.50	<2.50	<2.50	4.00	<2.50	4.00	10.00	<2.50	4.00	10.00	6.50	Rejected

[a] Rejected as an outlier by Dixon's test.

Table 6
Authentic data sets tested by inspection by variables

Project	Drug	Calibration cuve range (ng ml $^{-1}$)	Technique	Processing	Facility	% of runs accepted at AQL (120–80%)					% Runs rejected [a]	% of runs accepted at AQL(115–85%)					% Runs rejected [a]
						2.50	4.00	6.50	10.00	15.00		2.50	4.00	6.50	10.00	15.00	
A	A	1–250	LC/FLU	R	I	95	95	95	95	100	0	86	86	91	95	95	5
B	B	3–5000	LC/UV	R	I	100	100	100	100	100	0	100	100	100	100	100	0
C	C	1–60	LC/EC	M	A	100	100	100	100	100	0	71	71	100	100	100	0
D	D	1–200	LC/UV	M	I	78	78	89	89	89	11	55	55	55	55	67	33
E	E	1–250	LC/UV	M	I	78	78	89	100	100	0	78	78	78	78	100	0
F	F	0.15–100	LC/FLU	M	I	92	92	100	100	100	0	58	75	83	83	92	8
G	G	0.1–100	LC/FLU	R	I	100	100	100	100	100	0	100	100	100	100	100	0
H	D	0.1–100	GC/MS	M	I,B	92	92	92	100	100	0	75	83	92	92	100	0
I	H	5–1000	LC/UV	M	C	100	100	100	100	100	0	85	85	85	100	100	0
J	H	2.5–1000	LC/MS	M	C	63	68	87	97	97	3	45	47	58	63	82	18
K	E	4–1000	LC/UV	R	I	100	100	100	100	100	0	100	100	100	100	100	0
L	E	1–250	LC/UV	M	D	92	92	100	100	100	0	85	85	92	92	100	0
M	B	3–5000	LC/UV	M	A	83	83	100	100	100	0	83	83	83	83	100	0
N	I	1–100	GC/MS	M	C	94	94	100	100	100	0	88	88	88	94	100	0
Mean	–	–	–	–	–	91	91	97	99	99	1	79	81	86	88	95	5

Key: R, samples processed by robot; M, samples processed manually; I, samples assayed internally at Glaxo; A, B, C, D, samples assayed at contract laboratory A, B, C, or D; LC/FLU, samples assayed by liquid chromatography with fluorescence detection; LC/UV, samples assayed by liquid chromatography with uv detection; LC/EC, samples assayed by liquid chromatography with electrochemical detection; GC/MS, samples assayed by gas chromatography with mass spectrometric deteection.

[a] Refers to an AQL of 15%.

It is well known that variability in the pharmacokinetic parameters is rather large and that the coefficient of variation may be as high as 100% and more. Such a process may warrant AQL and specification at 120–80%, while some other much more reproducible parameter may require AQL of 2.5% and specifications of 115–85%. Inspection by variables is flexible enough to accommodate specifications of various projects and permits the design of consistent, project-specific acceptance criteria.

The system is very flexible. With the advent of highly productive analytical technologies, such as LC–MS, batches of analytical samples may become very large, even up to 300–400 samples. Inspection by variables is flexible enough to provide for these changes and offers ready answers regarding the number of QCs that should be used and what the acceptance criteria should be.

It should be also noted that inspections by variables also handles satisfactorily much smaller batches, in which the routine use of six QCs is not warranted, but three or four may be appropriate.

This approach may also be used as a tool in deciding the price for an assay at a control laboratory. Lower AQLs may command higher prices, if needed, while higher AQLs may warrant lower prices. Should productivity and sample throughput be the main concern, their AQL and/or specification limits may be increased. All of this could be achieved within one and the same statistical system, avoiding arbitrary, inconsistent decisions.

One may notice that the method performance during its validation is not mentioned here and acceptance criteria are not based on the validation results. In the opinion of this author, it is a matter of analytical philosophy whether or not a link needs to be made between the method validation results and the execution of a bionalytical project. This author believes that the role of the validation is to prove that the method/analyst/equipment is able to meet or surpass specified criteria of precision, accuracy, specificity, etc. With this proof in hand the acceptance criteria for a bioanalytical project should be based solely on the specification.

One strategy of the inspection by variables, the use of switching rules, is not recommended here for practical reasons. Switching from normal to tightened inspection is recommended when two out of five successive batches are rejected. Returning from the tightened to normal inspection occurs when five consecutive batches are accepted at tightened inspection [10]. Bioanalytical projects are frequently too small to fully benefit from the switching rules, as a project may be finished after 6–8 runs. Also, the switching rules serve as a pressure tactic, which is unnecessary today as the competition between the internal laboratory and several external laboratories may be used to drive the quality. Hence, it is probably better to accept the normal or tightened inspection for the whole project and avoid confusion by having different sets of rules.

5. Conclusions

Inspection (sampling) by variables is a valid, internally coherent tool in accepting results in bioanalysis. It provides immediate answers, it is based on internationally recognized principles (ISO 3951 and MIL-STD-414), it incorporates precision, accuracy, and specification into the acceptance criteria, and it relates batch size with the sample size. For the Conference Report recommendation to be in agreement with ISO 3951, an AQL of 6.50% or lower must be selected. The flexibility of this system permits the accommodation of larger and smaller batches of samples, and establishes statistically valid project-specific acceptance criteria. It is proposed that the fixed range acceptance criteria be replaced with inspection by variables for bioanalysis performed as an application in bioavailability, bioequivalence and pharmacokinetic studies.

Acknowledgment

Author thanks Julie Tomlinson for her assistance in editing the paper.

References

[1] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman and S. Spector, J. Pharm. Sci., 81 (1992) 309–312.
[2] A.C. Cartwright, Drug Inf. J., 25 (1991) 471–482.
[3] H.T. Karnes and C. March, Pharm. Res., 10 (1993) 1420–1426.
[4] J.R. Lang and S. Bolton, J. Pharm. Biomed. Anal., 9 (1991) 435–442.

[5] C. Hartmann, D.L. Massart and R.D. McDowall, J. Pharm. Biomed. Anal., 12 (1994) 1337–1343.
[6] A.G. Causey, H.M. Hill and L.J. Phillips, J. Pharm. Biomed. Anal., 8 (1990) 625–628.
[7] R. Kringle, Pharm. Res., 11 (1994) 556–560.
[8] J.M. Juran, Quality Control Handbook, 3rd edn., McGraw-Hill, New York, 1979.
[9] A.J. Duncan, Quality Control and Industrial Statistics, 4th edn., Richard D. Irwin, Homewood, Il, 1974.
[10] G.B. Wetherill and D.W. Brown, Statistical Process Control, Chapman and Hall, London/New York/Tokyo/Melbourne/Madras, 1991.
[11] Sampling Procedures and Tables for Inspection by Variables for Percent Defective, MIL-STD-414, U.S. Department of Defense, Military Standard, Government Printing Office, Washington, DC, 1957.
[12] Sampling Procedures and Charts for Inspection by Variables for Percent Nonconforming, ISO 3951-1989, International Organization for Standardization, CH-1211 Geneva 20, Switzerland, 1989.
[13] R. Caulcutt, Statistics in Research and Development, 2nd edn., Chapman and Hall, London/New York/Tokyo/Melbourne/Madras, 1991.