Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 906–913
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Is HPLC assay for drug substance a useful quality control attribute?

Jeffrey D. Hofer, Bernard A. Olsen *, Eugene C. Rickard

Analytical Sciences Research & Development, Eli Lilly & Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 21 February 2007; received in revised form 18 April 2007; accepted 19 April 2007
Available online 24 April 2007

Abstract

HPLC is a generally accepted method for assay of drug substances. However, recent claims cast doubts on the utility of HPLC assay methods for characterizing quality [S. Görög, J. Pharm. Biomed. Anal. 36 (2005) 931–937]. This study examines the utility of the traditional drug substance HPLC assay as a quality control parameter. HPLC assay data from more than 100 batches for each of eight drug substances were compared to results from a mass balance approach (100 − impurities%). Estimates of the variability of HPLC assays from our data and from the literature ranged from 0.6 to 1.1% R.S.D. This variability is an appreciable portion of a typical acceptance range (e.g., 98.0–102.0%) and frequently exceeds the variability of the manufacturing process. Therefore, the results of the HPLC assay are questionable at best to determine the acceptability of the drug substance batch. The high variability also can generate a significant percentage of false out-of-specification (OOS) results, even when the "true" purity is 99.0–100.0%. Each false OOS leads to inefficiencies because of unwarranted investigations for a root cause and/or implementation of countermeasures for a problem that does not exist. Lastly, low precision makes it nearly impossible to detect significant changes in the process mean and/or degradation during a stability study. The use of a mass balance approach for assay retains essentially the same average results as the HPLC assay but gives standard deviations that are up to 10 times less. Monitoring the assay by mass balance allows for more precise process and stability monitoring and facilitates more rapid and accurate identification of process changes.

Keywords: HPLC assay; Drug substance; Assay variability; Out-of-specification; Precision

1. Introduction

The goal of pharmaceutical manufacturers is to produce drug substance of acceptable quality for formulation into a drug product. An array of tests is used to determine if the material has acceptable quality prior to formulation of the drug product. Assay by HPLC is one of the most common chemical tests used to measure the quality of the drug substance. The utility of HPLC for this use is impacted by the precision that can be achieved. Görög [1] estimated the precision of compendial HPLC methods to be in the range of 0.5–1.0%. A literature review of typical intermediate precision values for HPLC assays shows ranges of about 0.2–1.7% with averages between 0.6 and 1.1% [2–4]. This paper examines HPLC assay precision estimates for many drug substances in order to substantiate these estimates.

The inherent variability of the HPLC assay results in three major limitations for its capability to monitor drug substance quality. One limitation is the problematic ability to accurately determine if the drug substance batch meets the regulatory acceptance criterion, especially when the acceptance criterion is a narrow range such as 98.0–102.0%. Bunnell [5] summarized this situation as "that when a reasonable scheme is employed, the expected uncertainty of the result approaches the magnitude of the entire span of the typical drug substance specification, and it would be a mistake to rely too heavily on an assay result alone as proof of drug substance purity." A second consequence of low precision occurs when any result falls outside of the regulatory acceptance criteria. An out-of-specification (OOS) investigation must be performed to identify the root cause followed by taking appropriate corrective and preventative action [6–8], which results in wasted resources if the true cause of the OOS is analytical variability rather than a failure to meet quality standards. A third limitation of an assay by HPLC is its inadequate ability to effectively discriminate between drug substance batches of different quality [1]. It is imperative that a change in the quality of manufactured material be detected as soon as possible to maintain control of the manufacturing process. In summary, the limitations ensuing from the typical precision of the HPLC assay

* Corresponding author. Tel.: +1 317 651 9910; fax: +1 317 433 7477.
E-mail address: olsen_bernard_a@lilly.com (B.A. Olsen).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.04.023

of approximately 0.6–1.1% arise because the assay variability is an appreciable portion of the acceptance range, frequently exceeds the variability of the manufacturing process, and may produce false OOS results.

These failures of assay by HPLC to achieve the desired discrimination in quality can be circumvented by using a combination of a more precise non-specific assay, such as titration or UV, in combination with an appropriate technique, such as HPLC, to measure impurities [9–10]. However, HPLC is usually chosen in spite of its limitations because of the preference for a single specific, stability-indicating technique to quantitate the drug substance [10] and the operational simplicity and efficiency for the laboratories.

Because of the limitations of the HPLC assay, which are verified by the data analysis in this article, a mass balance approach to evaluate purity is proposed. This article compares the relative capability of the proposed mass balance purity to the capability of the HPLC assay to determine if the drug substance batch meets the regulatory acceptance criteria, monitor the manufacturing process and identify changes in quality. HPLC assay data from more than 100 batches for each of eight drug substances are presented to demonstrate the capability of the mass balance evaluation of purity. This article also evaluates the probability of obtaining false out-of-specification results due solely to measurement variability.

2. Objectives of drug substance HPLC assay for quality control, typical acceptance criteria and process variability

HPLC assays are intended to monitor the release and stability of the drug substance with high specificity, high accuracy and the best possible precision. Other non-specific methods may be more precise than the HPLC assay but are not as useful for monitoring batch release and stability because they usually have positive interferences from related compounds. Ideally, the drug substance assay will:

- Ensure that a high quality drug substance has been produced.
- Accurately determine if the drug substance meets the regulatory acceptance criterion.

Typical regulatory expectations for the drug substance acceptance criterion for assay by HPLC are 98.0–102.0%. In some cases, the acceptance criteria are even narrower. Examples of narrow acceptance ranges for assay by HPLC from several USP drug substance monographs [11] are given in Table 1. These narrow acceptance criteria found in pharmacopoeial monographs may contribute to the temptation of regulatory agencies to push for narrow assay acceptance criteria in a misguided effort to control purity.

It is important that the drug substance manufacturing process remains in control, that is, that the process produces material of consistent quality. Thus, it is important to immediately determine when unexpected changes in quality occur so that appropriate investigations and countermeasures can be undertaken. For a synthetic drug substance of high purity (98–100%), a change of 0.5% in purity would be considered to be a major change and one would like to detect this with high certainty as soon as possible following the change.

Table 1
Examples of HPLC assay acceptance criteria in USP

Drug substance	Drug substance HPLC assay acceptance criteria (%)
Dorzolamide hydrochloride	99.0–101.0
Lovastatin	98.5–101.0
Indinavir sulfate	98.5–101.5
Lansoprazole	99.0–101.0
Tacrine hydrochloride	98.5–101.5

3. Typical drug substance HPLC assay intermediate precision

For control methods in general, the smaller the variability of the analytical method relative to the acceptance criteria range and the manufacturing variability, the better the method. Conversely, the higher the variability is relative to these ranges, the less useful the analytical method becomes as a monitor of quality. The typical intermediate precision observed in our laboratories for several HPLC assay methods was examined to evaluate the usefulness of the HPLC assay for assessment of quality.

Intermediate precision estimates for several internal drug substance HPLC assay methods are given in Table 2. All methods involve similar sample preparation steps including weighing a dry powder, dissolving and diluting. The methods typically utilized duplicate or triplicate preparations of standard and sample solutions. These data were obtained from two types of studies. Design of experiments (DOE) techniques were used to study robustness with respect to different analysts, instruments, columns and days. Intermediate precision data are readily obtained from such studies. The other type of study, labeled "Control Sample", was conducted by analyzing the same batch of a given compound each time a set of samples was analyzed. Although not a statistically designed study, the control sample approach gives a realistic picture of intermediate precision because it incorporates many typical sources of variability encountered during actual use of the method. Control sample studies were included only for drug substances known to be chemically stable to prevent biased estimates of variability caused by sample degradation. Note that the control sample and most of the DOE studies were performed in a single laboratory and, therefore, do not capture lab-to-lab variability (ICH reproducibility).

The intermediate precision data in Table 2 (pooled R.S.D. = 0.61, range = 0.29–1.0%) are consistent with previously reported findings. Görög [1] described that "a cautious estimate for the precision of compendial HPLC methods can be characterized by R.S.D. of about 0.5–1%." Ermer and Ploss [3] observed a 1.05% average R.S.D. intermediate precision/reproducibility for seven drug substances (range = 0.35–1.68%). In another article, Ermer et al. [4] gave a range of 0.5–1.1% with an average of 0.86% for the intermediate

Table 2
Intermediate precision estimates for HPLC assay of drug substances

Drug substance	Study type[a]	*N*	R.S.D. (%)
A	Control sample	53	0.77
B	DOE	48	0.67
C	DOE	24	0.60
D	Control sample	89	0.64
E	Control sample	16	0.29
F	Control sample	38	0.56
G	Control sample	31	0.61
H	Control sample	65	0.66
I	Control Sample	15	0.79
J	DOE	12	0.54
K	DOE	12	0.51
L	Control sample	41	0.64
M	DOE	48	0.47
N	Control sample	67	1.0
O	DOE	18	0.8
P	Control sample	22	0.46
Q	Control sample	47	0.32
R	Control sample	49	0.53
S	Control sample	69	0.45
T	Control sample	43	0.53
U	Control sample	112	0.37
		d.f. = 898	Pooled R.S.D. = 0.61; range = 0.29–1.0

[a] DOE: design of experiments and d.f.: degrees of freedom.

precision from nine drug substance stability studies with 217 values. Based on system performance data, Renger [2] estimated HPLC assay intermediate precision to range from 0.6 to 0.8% for an automated HPLC system, in very close agreement with results in Table 2, and slightly higher, 1.1–1.5%, for a typical HPLC system. Bunnell [5] simulated the precision of HPLC assay results using estimates of variability contributions from sample preparation, injection and peak detection and integration. The sample and injection replication scheme used for analysis was incorporated in the simulation. Within-day precision estimates of 0.92 and 0.41% were calculated for replicate preparation/replicate injection schemes of 1/1 and 3/2, respectively. Although these estimates do not include day-to-day variability, they are consistent with results given in Table 2.

4. Implications of intermediate precision for use of HPLC assay

Results in Table 2 and a review of previous reports demonstrate that the typical intermediate precision R.S.D. of the drug substance HPLC assay is approximately 0.6–1.1%. Thus, an acceptance criterion with a range of only ±2.0% is very narrow relative to the inherent method variability. While the common practice of establishing acceptance criteria that are three times the intermediate precision may appear to give an appropriate range for the specification, there is a non-negligible chance of observing false OOS results even if the process mean remains constant. In addition, the low probability of a false OOS for an individual result rapidly becomes a cumulative, non-negligible probability for observing one OOS as the number of observations increase as discussed later in this article. The recent US Food and Drug Administration (FDA) OOS guidance [6] raises more concerns because it requires that all individual sample replicates, as well as the average, fall within the acceptance criteria (Section 5(B) of guidance).

Therefore, the "true" value for the HPLC assay must remain within a very narrow range or else an unacceptably high frequency of OOS results will occur when monitoring drug substance manufacturing. That is, the observed HPLC assay results will vary over the greater part of the acceptance criteria range due solely to the inherent variability of the HPLC assay. Likewise, observed results for the HPLC assay during a stability study will cover most of the acceptance criteria range when the drug substance, in fact, is stable as shown by little or no change in related substance content.

4.1. Potential for a false out-of-specification result for a given batch

With narrow acceptance ranges, the potential for a false OOS result is very dependent on the true batch value and the method precision. Table 3 shows the probability of observing a false OOS result for different true batch means when the HPLC assay standard deviation (S.D.) is 0.6, 0.8 or 1.0 and the acceptance criterion is 98.0–102.0%. The chance of obtaining a false OOS increases quite quickly as the standard deviation increases (for example, only a 1% chance when S.D. = 0.6 and mean = 99.4 but nearly a 9% chance when S.D. = 1.0 for the same mean). This suggests that the HPLC assay is more a test of the laboratory's ability to achieve high precision than of drug substance quality. Of course, the probability of false OOS also increases, as the true result gets closer to the lower acceptance criterion.

The chance of observing a false OOS by chance also is relatively high when a lot is repeatedly tested (e.g., during stability

Table 3
Probability of observing an OOS result for a single test result with an acceptance criterion of 98.0–102.0%

True mean	Probability of observing OOS result when S.D. = 0.6	Probability of observing OOS result when S.D. = 0.8	Probability of observing OOS result when S.D. = 1.0
99.8	0.0015	0.0152	0.0498
99.7	0.0024	0.0188	0.0553
99.6	0.0039	0.0241	0.0630
99.5	0.0062	0.0313	0.0730
99.4	0.0098	0.0406	0.0854
99.3	0.0151	0.0525	0.1003
99.2	0.0228	0.0670	0.1176
99.1	0.0334	0.0847	0.1375
99.0	0.0478	0.1057	0.1600
98.9	0.0668	0.1303	0.1850
98.8	0.0912	0.1587	0.2125
98.7	0.1217	0.1908	0.2424
98.6	0.1587	0.2266	0.2746

studies) and the mean does not change. This situation is explored in the following example. Assume the batch mean is 99.6 and the standard deviation of the HPLC assay is 0.6, 0.8 or 1.0. For any one instance, the chance of observing a false OOS is low (0.4, 2.4 and 6.3%, respectively) as shown in the Table 4. However, as the number of test results increase, the likelihood of observing at least one false OOS increases. For example, if only 10 tests are performed, the corresponding chances of obtaining one false OOS result for the same mean and standard deviations rise to about 4, 22 and 48%, respectively.

It is clear that even if a process mean remains stable and/or a batch on stability does not change and the "true" value is well within the acceptance criteria, it is likely that individual false OOS results will be observed within a surprisingly small number of tests. This will result in unproductive investigations and countermeasures because there is truly nothing to find. That is, the process is in control and/or a product on stability is not changing and the batch truly meets specifications.

Table 4
Probability of observing an OOS result for repeated testing with an acceptance criterion of 98.0–102.0%

Number of tests	Probability of at least one false OOS result when each mean is 99.6 and S.D. = 0.6	Probability of at least one false OOS result when each mean is 99.6 and S.D. = 0.8	Probability of at least one false OOS result when each mean is 99.6 and S.D. = 1.0
1	0.004	0.024	0.063
2	0.008	0.048	0.122
3	0.012	0.071	0.177
4	0.015	0.093	0.229
5	0.019	0.115	0.278
6	0.023	0.136	0.323
7	0.027	0.157	0.366
8	0.030	0.177	0.406
9	0.034	0.197	0.443
10	0.038	0.216	0.478
15	0.056	0.306	0.623
30	0.110	0.519	0.858
50	0.176	0.705	0.961
100	0.321	0.913	0.999

The above probabilities apply to the final reportable value of the analytical method. If the analytical method reportable value is defined as the average of more than one replicate, the likelihood of an OOS result is even higher because of the FDA OOS guidance expectation that all of the individual replicates also must fall within the acceptance criteria.

Laboratories can reduce the risk of false OOS results by taking steps to minimize method variability. These steps can include incorporating data acceptance criteria for sample replicate precision (not system suitability) into the method, increasing the number of replicates, or using a limited number of analysts, instruments, etc. However, the generation of multiple replicate results to improve precision increases the probability of a false OOS given the FDA OOS guidance [6] expectation for each replicate. Some authors [12] have recommended that an increase in sample weight to greater than 160 mg may be sufficient to significantly reduce the variability of HPLC assays. While this may result in a minor reduction in variability, it may not be sufficient to achieve the desired method precision. Also, the larger solvent volumes and/or additional dilutions required for larger sample weights may not be practical and may reduce laboratory efficiency.

4.2. HPLC assay lacks the ability to rapidly identify large (0.5%) shifts in the mean

An illustration of the difficulty of the HPLC assay to quickly identify a change in quality is shown in the following example for a drug substance process:

- For the first 50 batches, the true assay mean is 99.5% with an HPLC assay standard deviation of 0.6%.
- Starting with batch 51, the true assay mean shifts 0.5% lower to 99.0% with the same HPLC assay standard deviation.

A change from 99.5 to 99.0% would be considered a large change in the process mean (perhaps due to a new process impurity) that would need to be investigated followed by implementation of appropriate countermeasures. However, Fig. 1 unmistakably shows that there is virtually no ability to detect a large change in quality from a single observation (i.e., the batch release result). Moreover, the simulation results in Fig. 1 clearly show that even after multiple batches have been manufactured following the process change, it is quite difficult to readily identify that a process shift has occurred. Furthermore, if one decides that a process shift actually occurred, it is difficult to determine when it happened to facilitate the investigation of the root cause.

The one-sided *t*-test, a common statistical test, can be performed to ascertain whether the purity mean has decreased. The difficulty of using the *t*-test to quickly identify a decrease in the purity process mean given the usual amount of assay variabil-

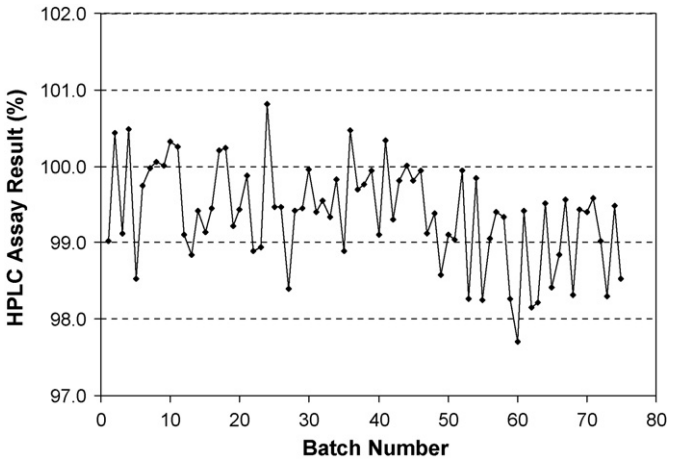

Fig. 1. Simulation of a 0.5% decrease in purity process mean at batch 50 with HPLC assay standard deviation of 0.6%.

ity is illustrated by the following example for a drug substance process:

- For the first 40 batches, the true assay mean is 99.8% with an HPLC assay standard deviation of 0.6%.
- Starting with batch 41, the true assay mean decreases 0.2, 0.4 or 0.6% to 99.6, 99.4 or 99.2%, respectively, with the same HPLC assay standard deviation.

A simulation was performed to determine how likely it would be for a one-sided *t*-test to identify that the results for 3–40 batches after the change had a statistically significant lower mean compared to the first 40 batches. The simulation used a false detection rate of 5%; that is, a 5% chance of falsely concluding that the process mean has decreased when it in fact has not.

Fig. 2 plots the probability of statistically detecting a decrease in the purity process mean for different numbers of batches produced following the decrease in the process mean. The plot

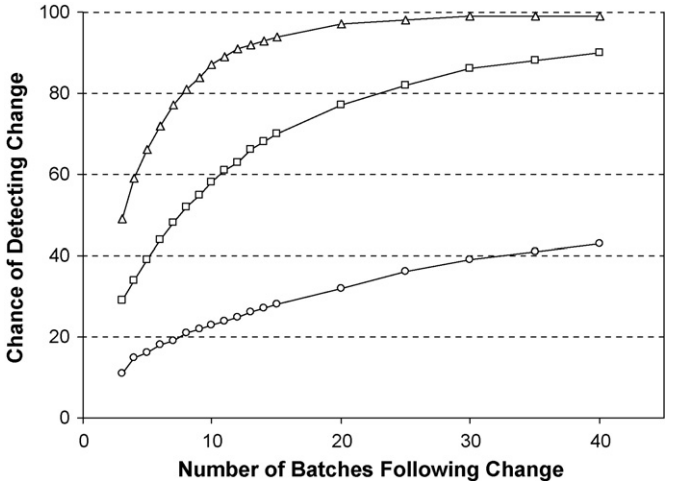

Fig. 2. Simulation of power to detect a decrease in purity process mean from 99.8% with one-sided *t*-test with $\alpha = 0.05$ when standard deviation = 0.6. Open triangles: post change mean = 99.2%; open squares: post change mean = 99.4%; open circles: post change mean = 99.6%.

demonstrates that a decrease of 0.6% takes more than 10 batches to have a 90% chance of detecting the decrease in the process mean. For a change of 0.4%, it takes more than 20 batches to have even an 80% chance of detecting the decrease. Finally, it is virtually impossible to reliably detect a decrease of 0.2%. The situation would be much worse for an intermediate precision of 1.1%, the upper range of observed R.S.D.s.

These examples show that the HPLC assay cannot distinguish between drug substance batches that are quite different in quality (e.g., mean difference of 0.5%) to detect decreases in the process purity, i.e., to determine if the process is "in-control". Görög [1] summarizes the above situation by stating "This means that the analytical error to be counted with is certainly above 0.5% and is probably around 1%. This makes the value of assay results obtained even by the highly specific HPLC methods as a means for characterizing the quality of bulk drug materials at least questionable". Bunnell's [5] work also led to the conclusion that "it is virtually impossible to distinguish between assay results that differ by less than 1%."

5. Mass balance assay approach as alternative to HPLC assay

As has been shown, the HPLC assay has several limitations for its ultimate utility for monitoring drug substance quality. The use of mass balance to calculate an assay result is an alternative approach that can provide a better way to accurately determine if the drug substance meets the regulatory acceptance criteria and to reliably detect unexpected changes in the quality of the drug substance [1]. Eq. (1) defines the mass balance estimate of batch purity, where all results are expressed as w/w percentages. The only requirement to implement this approach is that all impurities, including water and organic solvents, present in significant amounts are being measured accurately.

$$\text{Mass balance assay} = 100 - \text{related compounds} - \text{solvents} - \text{water} - \text{residue} - \text{other impurities} \quad (1)$$

Note that when related compounds are determined on a percent of total area basis rather than a weight percentage versus an external standard, the related compound result should be corrected by a factor of [(100 − solvents − water − residue − other impurities)/100] to give an accurate result. Unless the solvent and other impurity levels are relatively high, however, this correction will be small.

Figs. 3 and 4 are overlay plots of more than 150 batches of two drug substances where a mass balance assay was computed and compared to the HPLC assay result. These case studies illustrate how much more effective a mass balance assay approach is compared to the HPLC assay. Very similar process averages are obtained for the two approaches, but a dramatic reduction in variability is observed with the mass balance approach compared to the direct HPLC assay approach, which gives a more accurate evaluation of the process performance.

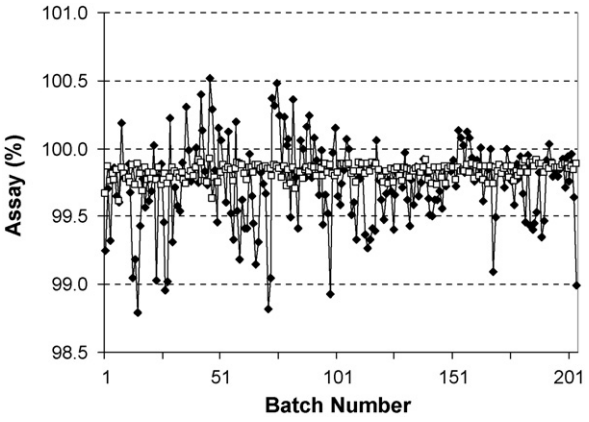

Fig. 3. Comparison of HPLC assay result to mass balance assay result for drug substance, example #1 (drug substance BB in Table 5). The HPLC assay results are represented by diamonds and the mass balance assay results are represented by open squares.

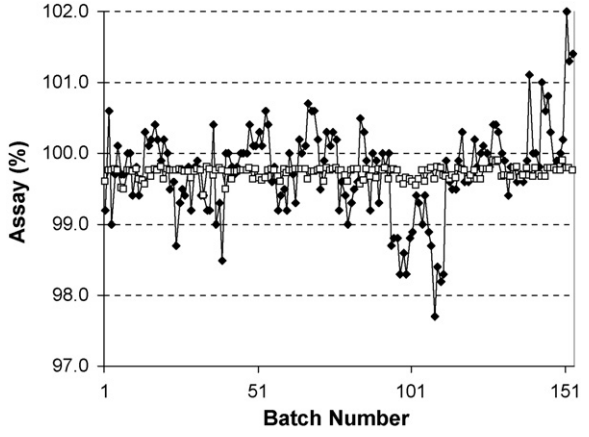

Fig. 4. Comparison of HPLC assay result to mass balance assay result for drug substance, example #2 (drug substance DD in Table 5). The HPLC assay results are represented by diamonds and the mass balance assay results are represented by open squares.

An evaluation was performed for several sets of internal drug substance assay results to estimate the reduction in variability obtained with a mass balance assay approach. As seen in Table 5, the standard deviation of the mass balance results in these examples is about 0.09% (range = 0.04–0.20%) or about 6- to 8-fold (range = 4–11) lower than that of the HPLC assay (pooled standard deviation 0.55%). Clearly there is a significant reduction in variability when using the mass balance approach while very similar averages are obtained.

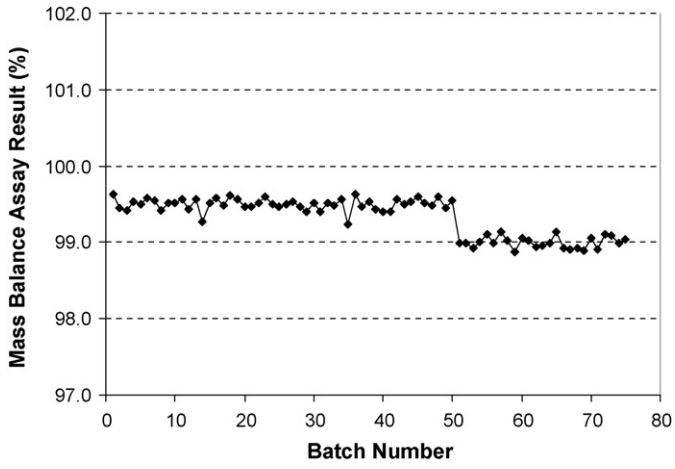

Fig. 5. Simulation of a 0.5% decrease in purity process mean at batch 50 with mass balance assay standard deviation of 0.08%.

5.1. Advantages of the mass balance assay approach

The mass balance assay offers much improved ability to detect shifts in the production process. Reconsider the previous example for a drug substance process but using the standard deviation of the mass balance assay:

- For the first 50 batches, the true assay mean is 99.5% with a mass balance assay standard deviation of 0.08%.
- Starting with batch 51, the true assay mean is lowered 0.5 to 99.0% with the same mass balance assay standard deviation.

Fig. 5 illustrates that by using a mass balance assay, it is easy to immediately identify that a decrease in purity has occurred and when it likely occurred. This ability to rapidly distinguish a purity decrease is in stark contrast to the previous example where a 0.5% decrease in purity would take at least 10 batches to sus-

Table 5
Summary of HPLC and mass balance statistics for drug substance assays

Drug substance	Number of batches	Mass balance assay average (%)	HPLC Assay average (%)	Mass balance standard deviation	HPLC assay standard deviation
AA	116	99.71	99.50	0.065	0.73
BB	204	99.83	99.73	0.055	0.31
CC	234	99.70	99.96	0.079	0.52
DD	153	99.72	99.75	0.080	0.64
EE	107	99.75	99.63	0.062	0.42
FF	107	99.63	100.07	0.042	0.42
GG	196	99.90	99.83	0.052	0.31
HH	111	99.66	100.36	0.20	0.80
	d.f.[a] = 1104	Pooled average = 99.75%	Pooled average = 99.85%	Pooled S.D. = 0.09; range = 0.042–0.20	Pooled S.D. = 0.55; range = 0.31–0.80

[a] d.f.: degrees of freedom.

pect that a decrease occurred and at least 20–30 batches to be confident of a purity decrease. Thus, the greater discrimination provided by the mass balance assay will lead to better decisions that will give a more consistent quality of drug substance. Note that the impurity results must be examined to determine if decreases in some impurities are balanced by increases in other impurities before concluding that there has been no change in quality.

The improved precision also will limit the likelihood of reacting to false OOS signals because the ability of the mass balance approach to discriminate between true process changes and random assay variation greatly exceeds that of the HPLC assay. The improved capability of a mass balance assay to detect quality differences eliminates a perceived need of regulatory agencies to push for narrow assay acceptance criteria as a means of controlling drug substance quality, an objective that cannot be met by HPLC.

Another advantage of a mass balance assay is the avoidance of shifts in HPLC assay results due to changes in the assignment of the reference standard purity. The assigned value for reference standard purity directly impacts the HPLC assay result. Changes in the average assay result caused by a shift in reference standard assignment can impact the ability to conform to tight acceptance criteria. Such shifts would not be observed with the mass balance approach.

A further benefit of the use of mass balance assay as opposed to HPLC assay could be realized when determining the amount of drug substance to use when formulating a batch of drug product. Because the mass balance assay value is much more precise than the HPLC assay value, the use of the mass balance result to determine how much drug substance to add to the drug product formulation will result in less variability around label claim for the drug product. This practice will give a more consistent drug product and lower the potential for OOS results for the drug product. In addition, the use of the mass balance result would avoid the logical inconsistency that arises when formulating the drug product using a drug substance HPLC assay result that is greater than 100.0%.

5.2. Is a mass balance acceptance criterion necessary?

The benefits of the mass balance approach over the HPLC assay to monitor drug substance quality are evident from the previous discussion. Although it may be tempting to simply add mass balance to the specification list as another analytical property with very narrow acceptance criteria, this is not necessary to achieve the desired benefits. Each of the constituents that contribute to the mass balance assay estimate is individually monitored with its own acceptance criterion and, therefore, no regulatory commitment for acceptance criteria should be necessary for mass balance. Mass balance gives an indication of overall impurity load rather than focusing only on individual impurities.

It is recommended that the mass balance assay be calculated and tracked internally to confirm that the drug substance meets regulatory acceptance criteria and detect any change in purity of a magnitude deemed critical to the process. That is, monitoring mass balance in addition to individual and total impurities, residual solvents and moisture can serve as a complement that provides better discrimination of product quality than the HPLC assay alone.

5.3. When would the HPLC assay still be needed?

While there are many benefits to the use of the mass balance assay result instead of the HPLC assay result, there are situations where the HPLC assay may still provide value. Some of the situations where the HPLC assay may be necessary are:

- When monitoring a process that is not well-controlled or degradation products are not known and where there is a likelihood that new impurities may not be detected by existing impurities methods. This would also apply to situations where contamination with materials extrinsic to the process could occur due to poor manufacturing practices.
- When a legally-binding public standard is needed, such as for compendial use (note that other techniques could also be used for this purpose).
- During initial process and method development to determine if all significant impurities are being detected by the impurity methods.
- For drug substances with complex impurity profiles or which rapidly degrade to multiple products that do not account for the decrease in assay due to lack of accurate response factors for all significant impurities.
- When purchasing material from third-party suppliers where the route of synthesis and manufacturing process has not been fully disclosed so knowledge of potential impurities may be incomplete.

6. Conclusions

In conclusion, the use of HPLC assays for monitoring the quality of drug substances is questionable at best. The typical precision of the HPLC assay (0.6–1.1%) is an appreciable portion of the typical acceptance range (98.0–102.0%) and frequently approaches or exceeds the variability of the manufacturing process. Therefore, it is nearly impossible to detect significant changes, such as 0.5%, in the process mean and/or changes in samples within a stability study. Changes only become apparent if they are extremely large or after an unacceptably high number of batches or timepoints have been tested. Likewise, the narrow acceptance criteria often established by regulatory agencies in combination with typical measurement variability have the potential to unnecessarily generate large numbers of false OOS results even when the "true" purity is 99.0–100.0%. Each false OOS leads to inefficiencies because of unwarranted investigations for a root cause and/or implementation of countermeasures for a problem that does not exist.

The use of a mass balance assay approach retains essentially the same average results as the HPLC assay but gives standard deviations that are up to 10 times less than that of the HPLC assay. Mass balance assay monitoring would allow for more

precise process and stability monitoring and facilitate more rapid and accurate identification of process changes.

Based upon the above, the authors believe that the mass balance assay is more capable than the HPLC assay of monitoring the production of high quality drug substances. The mass balance assay provides an improved ability to monitor the manufacturing process, determine conformance to the acceptance criteria and identify changes in the quality of the drug substance while minimizing the frequency of false OOS results. If an HPLC assay is still required for regulatory purposes, the acceptance limits for the results should be appropriate for the capability of the method. When the manufacturing process has been shown to be well-controlled and to consistently deliver material that meets acceptance criteria, a skip testing approach may be considered.

Acknowledgements

The authors would like to recognize Debra Achgill, Jill Ahern and Nathania Lahive for their assistance in compiling some of the data in this paper.

References

[1] S. Görög, J. Pharm. Biomed. Anal. 36 (2005) 931–937.
[2] B. Renger, J. Chromatogr. B 745 (2000) 167–176.
[3] J. Ermer, H.-J. Ploss, J. Pharm. Biomed. Anal. 37 (2005) 859–870.
[4] J. Ermer, C. Arth, P. De Raeve, D. Dill, H.-D. Friedel, H. Höwer-Fritzen, G. Kleinschmidt, G. Köller, H. Köppel, M. Kramer, M. Maegerlein, U. Schepers, H. Wätzig, J. Pharm. Biomed. Anal. 38 (2005) 653–663.
[5] R.D. Bunnell, Pharm. Res. 14 (1997) 156–163.
[6] US Dept. Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production, October 2006.
[7] ICH: Q7A, Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, November 2000.
[8] ICH: Q9, Quality Risk Management, November 2005.
[9] European Pharmacopoeia Technical Guide for the Elaboration of Monographs, fourth ed., Council of Europe, Strasbourg, 2005, p. 39.
[10] ICH: Q6A, Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, October 1999.
[11] United States Pharmacopeia, USP 29—NF 24, The USP Convention Inc., Rockville, MD, 2006.
[12] B. Dejaegher, M. Jimidar, M. De Smet, P. Cockaerts, J. Smeyers-Verbeke, Y. Vander Heyden, J. Pharm. Biomed. Anal. 42 (2006) 155–170.