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Do placebo based validation standards mimic real batch products behaviour? Case studies☆

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ABSTRACT

Analytical methods validation is a mandatory step to evaluate the ability of developed methods to provide accurate results for their routine application. Validation usually involves validation standards or quality control samples that are prepared in placebo or reconstituted matrix made of a mixture of all the ingredients composing the drug product except the active substance or the analyte under investigation. However, one of the main concerns that can be made with this approach is that it may lack an important source of variability that come from the manufacturing process. The question that remains at the end of the validation step is about the transferability of the quantitative performance from validation standards to real authentic drug product samples.

In this work, this topic is investigated through three case studies. Three analytical methods were validated using the commonly spiked placebo validation standards at several concentration levels as well as using samples coming from authentic batch samples (tablets and syrups). The results showed that, depending on the type of response function used as calibration curve, there were various degrees of differences in the results accuracy obtained with the two types of samples. Nonetheless the use of spiked placebo validation standards was showed to mimic relatively well the quantitative behaviour of the analytical methods with authentic batch samples. Adding these authentic batch samples into the validation design may help the analyst to select and confirm the most fit for purpose calibration curve and thus increase the accuracy and reliability of the results generated by the method in routine application.

1. Introduction

Prior to the daily application of analytical methods in pharmaceutical laboratories for drug products release it is mandatory to validate them following for instance the ICH recommendations [1]. Analytical method validation usually involves validation standards or quality control samples that are prepared in placebo or reconstituted matrix made of a mixture of all the ingredients composing the drug product except the active substance [2–9]. However, one of the main concerns that can be made with this approach is that it may lack an important source of variability that could come from the manufacturing process when the final pharmaceutical formulation of the product is produced. This supplemental source of variability can result from the mixing, compressing, blending, heating, intensity of cohesion of particles, and so on, of all the ingredients including the active substance performed during production.

In the other hand, the ICH guideline (Q2R1) [1] recommends that "*precision should be investigated using homogeneous, authentic samples*", and adds that the use of artificially prepared samples must be used only "*if it is not possible to obtain a homogeneous sample*". Therefore, the question that remains at the end of the validation step is about the transferability of the quantitative performance from validation standards to real drug product samples as most of the times only artificially prepared samples are used such as the spiked validation standards.

The aim of this paper is to answer partially this question as it has never been done to our best knowledge. To achieve this, three case studies have been performed. First a UV spectrophotometric method dedicated to the quantification of metformin in tablets

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was validated using artificially prepared validation standards as well as real tablets coming from a single produced industrial batch. Second, an isocratic HPLC method dedicated to the quantification of methyl parahydroxybenzoate content in carbocestiene syrup pharmaceutical formulation was also validated using both spiked validation standards and authentic syrup obtained from the manufacturing process of three different batches. Finally, a gradient HPLC-UV method aiming at quantifying simultaneously valsartan and hydrochlorothiazide in tablets was also similarly studied.

2. Material and method

2.1. Chemicals and solvents

Metformin hydrochloride standard reference, methyl parahydroxybenzoate standard reference, valsartan standard reference and hydrochlorothiazide standard reference were supplied by the European Pharmacopoeia (Strasbourg, France). Acetonitrile of HPLC grade was purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was purchased from Acros Organics (Geel, Belgium). Sodium acetate of analytical grade was supplied from Sigma–Aldrich (Steinheim, Germany). Deionized water was generated from a Milli-Q water purifying system (Millipore, Watford, UK).

2.2. Apparatus

2.2.1. UV determination of metformin

Determination of metformin was performed with an Agilent UV–VIS 8453E double-beam spectrophotometer (Agilent, Palo-Alto, CA, USA) at a wavelength of 232 nm using purified water as blank.

2.2.2. HPLC determination of methyl parahydroxybenzoate

The HPLC system consisted in a LaChrom (Merck-Hitachi, Darmstadt, Germany) composed of a quaternary pump L-7100, an autosampler L-7200, an oven L-7360 and a DAD detector L-7455. The mobile phase consisted in a 33/67 (v/v) mixture of acetonitrile and an aqueous buffer at pH 5.0 of sodium acetate and water. The chromatographic isocratic separation was made with a Nucleosil C18 column (150 mm × 4.6 mm ID.; particle size: 5 μm; Macherey-Nagel, Hoerdt, France) and the UV detection was performed at 245 nm. The flow rate was set at 1.5 ml/min and 20 μl of each sample was injected onto the column. The analysis run time was of 15 min.

2.2.3. HPLC determination of valsartan and hydrochlorothiazide

The same HPLC system as employed for the determination of methyl parahydroxybenzoate was used. The mobile phase consisted in a mixture of acetonitrile and a phosphate buffer at pH 3.0 prepared with a 1 mM potassium dihydrogen phosphate solution in deionized water. The pH was adjusted to 3.0 with phosphoric acid. The chromatographic gradient separation was made with a Zorbax-SB C18 column (50 mm × 4.6 mm ID.; particle size: 1.8 μm; Agilent) and the UV detection was performed at 273 nm. The gradient program is given in Table 1. The flow rate was set at 0.8 ml/min and 5 μl of each sample was injected onto the column.

Table 1
Gradient program used for the HPLC-UV method dedicated to the simultaneous determination of valsartan and hydrochlorothiazide.

Time (min)	pH 3.0 phosphate buffer	Acetonitrile
0	80	20
8	80	20
10	55	45
30	55	45
31	80	20
40	80	20

2.3. Solutions

2.3.1. UV determination of metformin

a. Sample solution

About 1/5th of the mean weight of 20 tablets is accurately weighed and transferred into a 100 ml volumetric flask. It is dissolved in purified water and diluted up to the mark. Then this solution is further filtered and about 1/100 of this solution is diluted with purified water.

b. Calibration and validation standards

Calibration standards and validation standards were prepared at three concentration levels: 80, 100 and 120% of the nominal target concentration of active substance in the drug product. The calibration standards prepared in purified water were analysed in duplicates during three days. Each validation standard prepared in a reconstituted matrix of the formulation (or placebo) was analysed in triplicates during the same three days, except the 100% level that was replicated 6 times each day.

2.3.2. HPLC determination of methyl parahydroxybenzoate

a. Sample solution

An accurately weighed sample of about 1.0 g of syrup is transferred to a 100 ml volumetric flask and diluted up to the mark with methanol. The same single initial source of syrup was used for each replicate and in each series to avoid introducing additional variability potentially coming from the batch heterogeneity.

b. Calibration and validation standards

Calibration standards and validation standards were prepared at five concentration levels: 60, 80, 100, 120 and 140% of the target nominal concentration of methyl parahydroxybenzoate in the syrup formulation. The calibration standards prepared in methanol were analysed once during 3 days. Each validation standard prepared in a reconstituted matrix of the formulation (or placebo) was analysed in triplicates during the same three days, except the 100% level that was replicated 6 times each day.

2.3.3. HPLC determination of valsartan and hydrochlorothiazide

a. Sample solution

Twenty tablets are crushed and a mass equivalent to the mean mass of a tablet is accurately weighed, dissolved and diluted to 100.0 ml with a 65/35 (v/v) mixture of pH 3.0 phosphate buffer and acetonitrile. For each replicate and for each series of analysis the same single initial sample of twenty tablets was used for the method validation using authentic batch samples in order to avoid introducing additional variability that may arise from the batch heterogeneity.

b. Calibration and validation standards

Calibration standards and validation standards were prepared at five concentration levels: 80, 90, 100, 110 and 120% of the target nominal concentration of valsartan and hydrochlorothiazide in the pharmaceutical formulation. The calibration standards prepared in a 65/35 (v/v) mixture of pH 3.0 phosphate buffer and acetonitrile were analysed in triplicates during 3 days. Each validation standard prepared in a reconstituted matrix of the formulation (or placebo) was analysed in triplicates during the same three days.

2.4. Validation approach

The validation approach used is the accuracy profile methodology [7–10]. This methodology is based on the concept of total

measurement error or results accuracy, i.e. the simultaneous combination of the systematic error (measured by biases, i.e. method trueness) and of the random error (measured by RSDs, i.e. method precision) [1,7–12].

What is required at the end of the validation step is to give guarantees that each of the future results that the laboratory will obtain during routine analyses will be accurate enough. Therefore, to reach this objective, instead of a whole set of statistical tests, the accuracy profile methodology uses only one statistical decision methodology, namely a β-expectation tolerance interval for one way ANalysis Of Variance (ANOVA) model [7–10]. This interval represents a region where it is expected to find each future result with a defined probability β specified by the user [13]. The following β-expectation tolerance interval formula is used [14]:

$$[L, U] = \left[\hat{\mu} - k\hat{\sigma}_{IP}; \hat{\mu} + k\hat{\sigma}_{IP}\right] \quad (1)$$

where $\hat{\mu}$ is the mean of the results and k is calculated in order to have an expected proportion β of the population within this interval. The formula of this tolerance interval is given by:

$$k = Q_t\left(df, \frac{(1+\beta)}{2}\right)\sqrt{1 + \frac{J\hat{R}+1}{JL(\hat{R}+1)}} \quad (2)$$

$$\text{with} \quad df = \frac{(\hat{R}+1)^2}{(\hat{R}+(1/L))^2/(J-1) + (1-(1/L))/JL} \quad \text{and} \quad \hat{R} = \frac{\hat{\sigma}_B^2}{\hat{\sigma}_W^2}$$

where $Q_t(df, (1+\beta)/2)$ is the $(1+\beta)/2$th percentile of a Student distribution with df degrees of freedom. Then, the intermediate precision variance can be estimated using: $\hat{\sigma}_{IP}^2 = \hat{\sigma}_B^2 + \hat{\sigma}_W^2$. $\hat{\sigma}_B^2$ is the run-to-run variance and $\hat{\sigma}_W^2$ is the within-run or repeatability variance obtained with ANOVA methodology [15]. J is the number of run performed and L the number of replicates per run.

2.4.1. Using spiked placebo validation standards

When the previously described validation approach is applied at the different concentration levels of the spiked placebo validation standards, an accuracy profile can be drawn [7–10]. Indeed the β-expectation tolerance interval is computed at each of these concentration levels and the method bias is estimated by deducting to each mean value the concentration of the validation standard. This profile is then compared to "a priori" defined acceptance limits. In the remaining of this work, the acceptance limits were set at ±10% while the probability β is fixed at 95%.

2.4.2. Using authentic batch samples

The following authentic batch samples were analysed:

- For the UV determination of metformin: tablets coming from a batch were analysed 6 times during each of the same days of the validation standards.
- For the HPLC-UV determination of methyl parahydroxybenzoate: syrups coming from three different batches were all analysed 6 times during the same days of the validation standards.
- For the simultaneous determination of valsartan and hydrochlorothiazide by gradient HPLC-UV, tablets coming from the same batch were analysed in triplicates during the same days of the validation standards.

The results obtained from the authentic batch samples are also analysed using the same β-expectation tolerance interval for one way ANOVA model. Indeed, the authentic batch samples are analysed in several runs and in several replicates within runs. Then, to compute total error of the authentic batch samples, the method bias ($\hat{\delta}$) obtained form the spiked validation standards matching

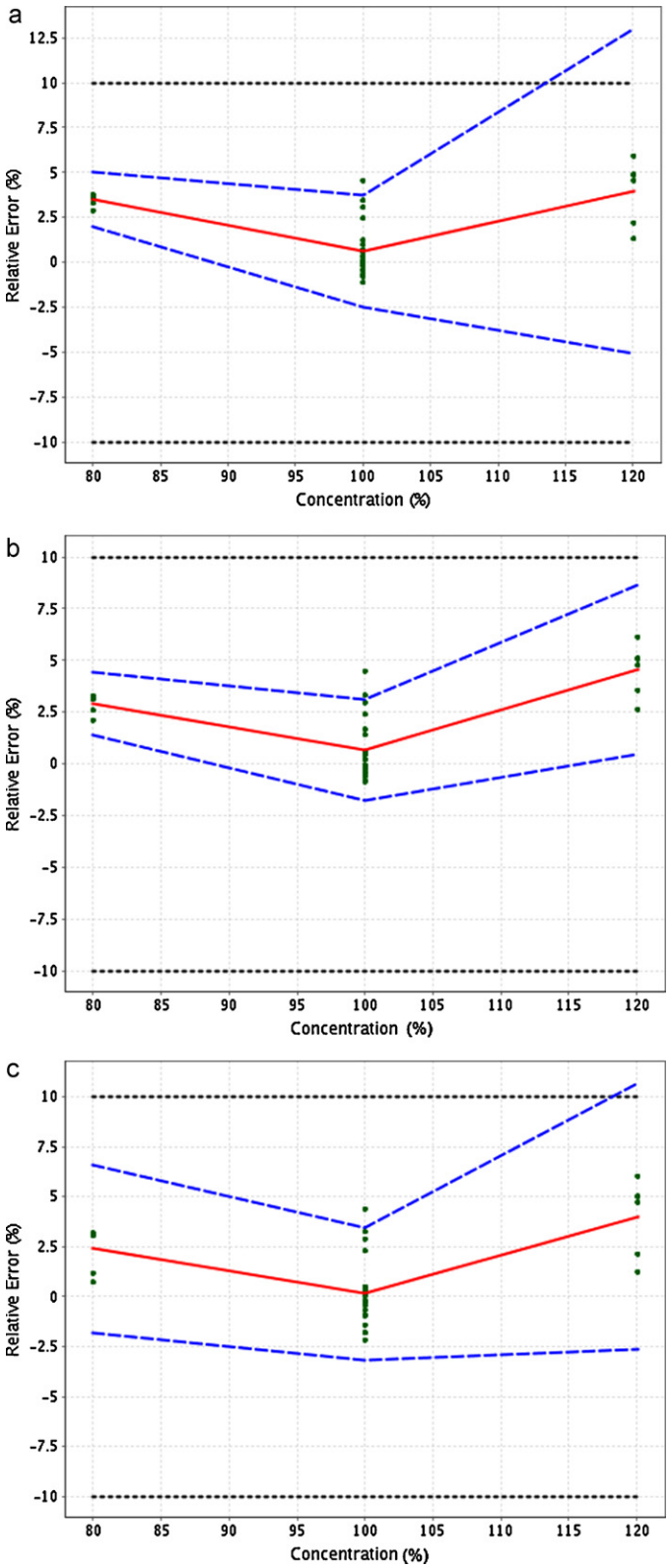

Fig. 1. Accuracy profiles of the UV method for the determination of metformin obtained for the spiked validation standards with the simple linear regression model (a) and with the linear model forced through the origin and fitted only with the 100.0% calibration level (b) or fitted only with the 120.0% calibration level (c). The plain line is the relative bias, the dashed lines are the 95% β-expectation tolerance limits and the dotted curves represent the acceptance limits (±10%). The dots represent the relative back-calculated concentrations of the validation standards.

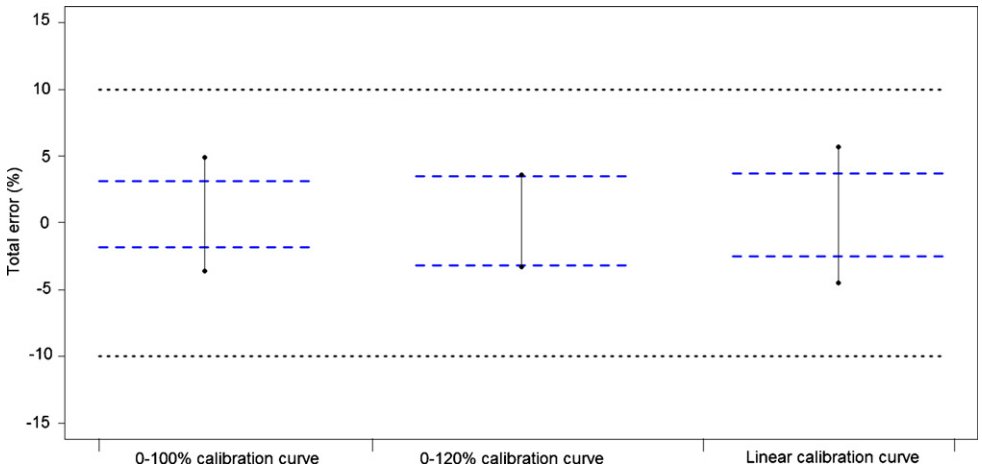

Fig. 2. Accuracy of the results obtained for the UV method for the determination of metformin for the authentic batch samples (tablets) with the simple linear regression model, with the linear model forced through the origin and fitted only with the 100.0% calibration level or fitted only with the 120.0% calibration level. The vertical continuous segments are the 95% β-expectation tolerance intervals obtained with the authentic batch samples, the dashed lines are the 95% β-expectation tolerance limits obtained with the 100% concentration level of the spiked validation standards and the dotted curves represent the acceptance limits (±10%).

the target nominal concentration of analyte under study in the drug product is added to this distribution and graphed.

2.5. Computations

All data treatments were realised using e-noval software V3.0 (Arlenda, Liege, Belgium) for the accuracy profile approach and the β-expectation tolerance intervals computations.

3. Results

3.1. UV determination of metformin

3.1.1. Spiked placebo validation standards

By using the approach proposed by Hubert et al. [8–10], the method is considered as valid within the range for which the accuracy profile is included inside the accuracy acceptance limits set at ±10%. This approach gives the guarantee that each future result generated by the method will be included within β-expectation tolerance limits with a user defined guarantee. Here this guarantee is set at 95.0%.

Three calibration models were investigated, namely the simple linear regression, the linear regression forced through the origin (0) and fitted only with the 100% calibration level or fitted only with the 120% calibration level. Accuracy profiles obtained with these calibration curves for the validation standards are shown in Fig. 1.

As illustrated in this figure, the method is thus considered valid over the whole concentration range investigated using a calibration curve forced through the origin and fitted only with the 100% level of the calibration standards as the 95% tolerance intervals are fully included within the ±10% acceptance limits (Fig. 1b). All the validation results obtained with this calibration curve are given in Table 2.

3.1.2. Authentic batch samples effect

In order to assess the impact of the process on the results accuracy, 95% β-expectation tolerance intervals were obtained from results of the real samples coming from a manufactured batch of tablets. The same previous calibration models were tested. Fig. 2 shows added to the β-expectation tolerance intervals computed for the real batch samples those obtained from the 100% concentration level of the spiked placebo validation standards. The β-expectation tolerance intervals were computed with the same guarantee: β = 95%.

As shown in Fig. 2, there is almost no difference between the tolerance intervals computed for the real samples (tablets) with the simple linear calibration model and the one forced through the origin and only the 100% calibration level. However, for these calibration models, the method seems more variable when analysing real tablets than when analysing spiked validation standards (Fig. 2). Nonetheless the quality of the results generated remains acceptable. Indeed, the tolerance intervals are still within the ±10% acceptance limits, thus corroborating the conclusion made with the spiked placebo validation standards. The results of precision and accuracy obtained with the calibration curve fitted only with the 100% calibration level for the authentic batch samples are given in Table 3.

When using the linear model forced through the origin and fitted only with the 120% calibration level it can be seen in Fig. 2 that the method precision for authentic batch samples is increased. Furthermore for this calibration curve the quality of the results generated when analysing real tablets and spiked placebos are equivalent. However it has to be reminded that when using the spiked placebo validation standards, this calibration curve did not allow to obtain accurate results at the highest concentration level, thus impairing the validity of this method when referring to the ICH Q2 requirements about the range criterion [1].

3.2. HPLC determination of methyl parahydroxybenzoate

3.2.1. Spiked placebo validation standards

The accuracy profile approach [8–10] has also been used to evaluate the validity of the HPLC-UV method for the determination of methyl parahydroxybenzoate in syrup. The accuracy acceptance limits were also set at ±10% and the minimum probability to obtain each future result generated by this method within the ±10% acceptance limits is set at 95.0%. Three calibration models were investigated. They are the simple linear regression, the linear regression forced through the origin (0) and fitted only with the 100% calibration level or fitted only with the 140% calibration level. Accuracy profiles obtained with these calibration curves for the validation standards are shown in Fig. 3.

All three calibration curves allow the method to be considered as valid over the whole concentration range investigated. Indeed, the 95% tolerance intervals are fully included within the ±10% acceptance limits in all cases. The final calibration curve selected was thus the calibration curve forced through the origin and fitted only with the 140% level of the calibration standards as it is one of the

Table 2
Validation results obtained with the spiked placebo validation standards for the UV method for the determination of metformin, for the HPLC-UV method for the determination of methyl parahydroxybenzoate as well as for the HPLC-UV method for the simultaneous determination of valsartan and hydrochlorothiazide.

	Metformin	Methyl parahydroxybenzoate	Valsartan	Hydrochlorothiazide
Calibration curve	0–100% linear model	0–140% linear model	0–120% linear model	0–120% linear model
Trueness				
Concentration level (%)	Relative bias (%)	Relative bias (%)	Relative bias (%)	Relative bias (%)
60	–	0.5	–	–
80	2.9	0.5	0.1	0.0
90	–	–	0.2	0.9
100	0.7	−0.4	0.0	0.0
110	–	–	0.0	−0.7
120	4.6	−0.7	−0.4	−1.3
140	–	0.6	–	–

	Metformin	Methyl parahydroxybenzoate	Valsartan	Hydrochlorothiazide
Calibration curve	0–100% linear model	0–140% linear model	0–120% linear model	0–120% linear model
Precision				
Concentration level (%)	Repeatability/intermediate precision (RSD, %)	Repeatability/intermediate precision (RSD, %)	Repeatability/intermediate precision (RSD, %)	Repeatability/intermediate precision (RSD, %)
60	–	2.2/2.2	–	–
80	0.2/0.5	1.2/1.2	0.1/0.5	0.1/0.9
90	–	–	0.1/0.4	0.1/0.7
100	1.4/1.4	0.8/0.8	0.1/0.6	0.1/0.3
110	–	–	0.2/0.6	0.2/0.4
120	0.6/1.4	0.6/0.9	0.3/0.3	0.2/0.9
140	–	0.7/1.9	–	–

	Metformin	Methyl parahydroxybenzoate	Valsartan	Hydrochlorothiazide
Calibration curve	0–100% linear model	0–140% linear model	0–120% linear model	0–120% linear model
Accuracy				
Concentration level (%)	Relative 95% β-expectation tolerance interval (%)	Relative 95% β-expectation tolerance interval (%)	Relative 95% β-expectation tolerance interval (%)	Relative 95% β-expectation tolerance interval (%)
60	–	[−5.8; 6.8]	–	–
80	[1.4; 4.5]	[−3.0; 4.0]	[−2.4; 2.6]	[−4.5; 4.4]
90	–	–	[−1.8; 2.3]	[−2.7; 4.6]
100	[−1.8; 3.1]	[−2.8; 1.9]	[−3.0; 3.0]	[−1.6; 1.5]
110	–	–	[−2.5; 2.4]	[−2.0; 0.6]
120	[0.5; 8.6]	[−4.1; 2.7]	[−1.0, 0.2]	[−5.6, 3.0]
140	–	[−8.1, 9.3]	–	–

Table 3
Results obtained for the analysis of the authentic batch samples for the UV method for the determination of metformin, for the HPLC-UV method for the determination of methyl parahydroxybenzoate as well as for the HPLC-UV method for the simultaneous determination of valsartan and hydrochlorothiazide.

	Metformin	Methyl parahydroxybenzoate			Valsartan	Hydrochlorothiazide
Calibration curve	0–100% linear model	0–140% linear model			0–120% linear model	0–120% linear model
Precision	Batch 1	Batch 1	Batch 2	Batch 3	Batch 1	Batch 1
Repeatability (RSD, %)	0.7	0.4	0.7	0.9	0.6	0.4
Intermediate precision (RSD, %)	1.6	1.1	1.1	1.6	0.8	0.8
Accuracy						
Relative 95% *p*-expectation tolerance interval (%)	[−3.6; 4.9]	[−5.2; 4.3]	[−4.2; 3.3]	[−6.4; 5.5]	[−2.3; 2.2]	[−3.2; 3.1]

simplest calibration model, it provides the least bias and leads to the least extrapolation of results. All the validation results obtained with this calibration curve are given in Table 2.

3.2.2. Authentic batch samples effect

To evaluate if the spiked validation standard mimic and predict well the quality of authentic batch sample results, samples coming from three different batches were analysed. Samples of each batch were analysed during three different days and in six replicates. The same calibration models as those evaluated with the spiked placebo standards were tested. Fig. 4 shows the 95% β-expectation tolerance intervals obtained with the authentic batch samples together with those obtained from the results of the analysis of the 100% concentration level spiked placebo validation standards as well as the ±10% acceptance limits.

For each calibration model tested, a batch-to-batch variability can be observed, as illustrated by the various lengths of the 95% β-expectation tolerance intervals shown in Fig. 4. What is also visible in Fig. 4 is that the method is providing less accurate results when analysing authentic batch samples than when analysing spiked placebo validation standards. All the calibration models tested are nonetheless providing results of adequate accuracy and reliability as all 95% β-expectation tolerance intervals are included within the ±10% acceptance limits. In this example, the linear model forced through the origin and fitted only with the 140.0% calibration level is the model providing results from authentic batch samples analysis of the best accuracy. This fully confirms the selection of this model as calibration curve with the spiked placebo validation standards. The results of precision and accuracy obtained with this calibration curve for the

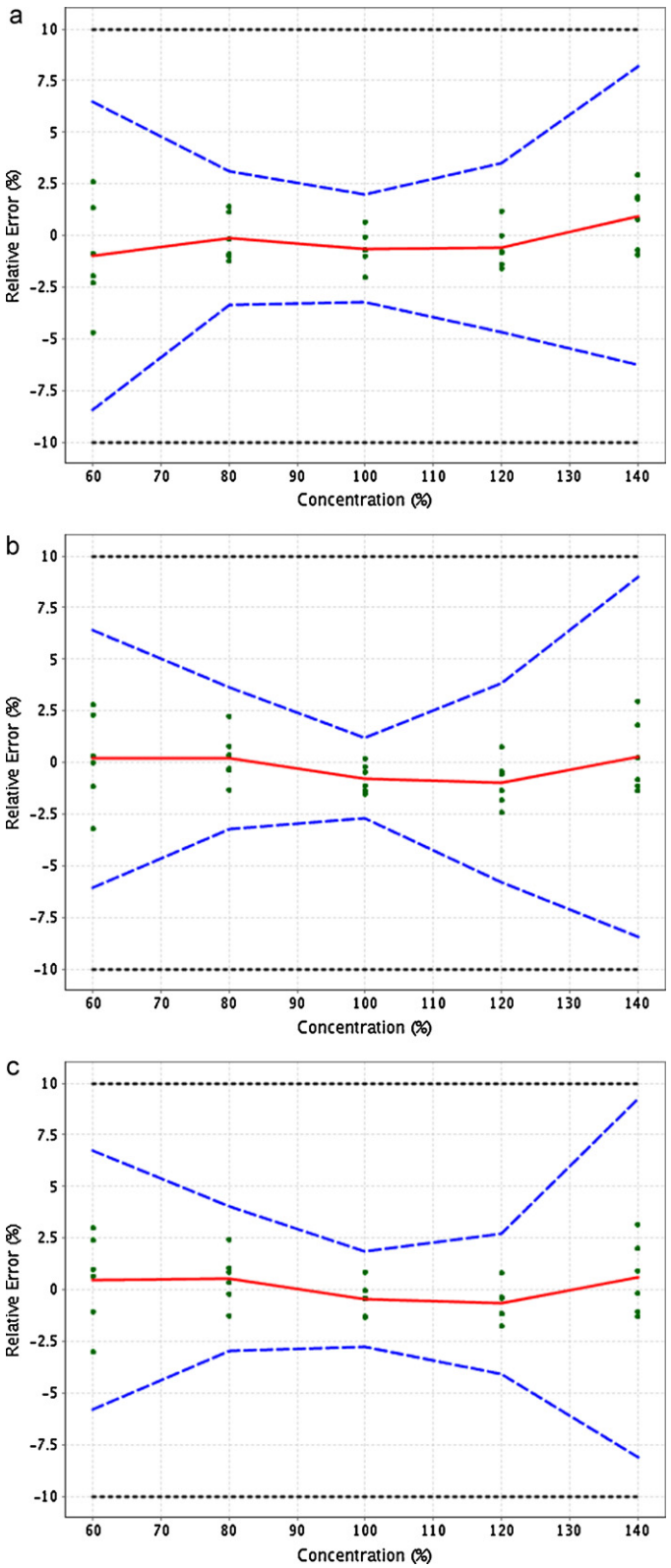

Fig. 3. Accuracy profiles of the HPLC-UV method for the determination of methyl parahydroxybenzoate obtained for the spiked validation standards with the simple linear regression model (a) and with the linear model forced through the origin and fitted only with the 100.0% calibration level (b) or fitted only with the 140.0% calibration level (c). The plain line is the relative bias, the dashed lines are the 95% β-expectation tolerance limits and the dotted curves represent the acceptance limits (±10%). The dots represent the relative back-calculated concentrations of the validation standards.

authentic batch samples are given in Table 3 for the three batches tested.

3.3. HPLC determination of valsartan and hydrochlorothiazide

3.3.1. Spiked placebo validation standards

For the validation of the HPLC-UV method for the determination of valsartan and hydrochlorothiazide in tablets, the accuracy profile approach was applied with acceptance limits also set at ±10% and the probability β is set at 95% for both analytes. The simple linear regression, the linear regression forced through the origin (0) and fitted only with the 100% calibration level or fitted only with the 120% calibration level were tested as standard curve. All these calibration curves allowed guarantee to obtain accurate results for both analytes over the whole concentration range studied. The final calibration curve selected was thus the calibration curve forced through the origin and fitted only with the 120% level of the calibration standards as it is one of the simplest calibration model, it provides the least bias and leads to the least extrapolation of results. All the validation results obtained with this calibration curve are given in Table 2 and the corresponding accuracy profiles for valsartan and hydrochlorothiazide are shown in Fig. 5a and b, respectively.

3.3.2. Authentic batch samples effect

To evaluate if placebo based validation standards mimic well the behaviour of authentic batch samples, tablets from a batch were analysed during three days and in triplicate. Fig. 6 shows the 95% β-expectation tolerance intervals obtained with the authentic batch samples together with those obtained from the results of the analysis of the 100% concentration level spiked placebo validation standards as well as the ±10% acceptance limits for both analytes and obtained with the previously selected calibration curve. As can be seen, the results obtained for valsartan from tablets are less variable than those obtained from the validation standards, while for hydrochlorothiazide it is the opposite behaviour. The results of precision and accuracy obtained with this calibration curve for the analysis of valsartan and hydrochlorothiazide in authentic batch samples are given in Table 3. Nonetheless, the 95% β-expectation tolerance intervals of both analytes obtained with the authentic batch samples are fully included into the ±10% acceptance limits, thus confirming the validity of the method and the selection of the calibration curve.

4. Discussion

From the three examples shown, it can be seen that adding the analysis of authentic batch samples as soon as they are available confirms and strengthens the decision about the validity of the method. Special care has been done when analysing the authentic batch samples to reduce as much as possible the increasing variability that may arise from the batches heterogeneity by using the same initial sample of tablets or syrups for one analysis to the other, especially for the two HPLC-UV methods. It therefore annihilates the increase in variability that may be due to the batch heterogeneity. While for the UV spectrophotometric method this was not done, the increase of variability that may originate from the batch heterogeneity is moderate. Nonetheless, some differences in the methods variability were observed between the uses of the two types of samples in the three case studies. Table 4 shows the p-values of the statistical tests of differences between the intermediate precision variance obtained for each type of sample for the three methods. As can be seen, they are all non-significant (p-values >0.05). This suggests that the differences observed are only due to the random sampling, and not coming from a special cause,

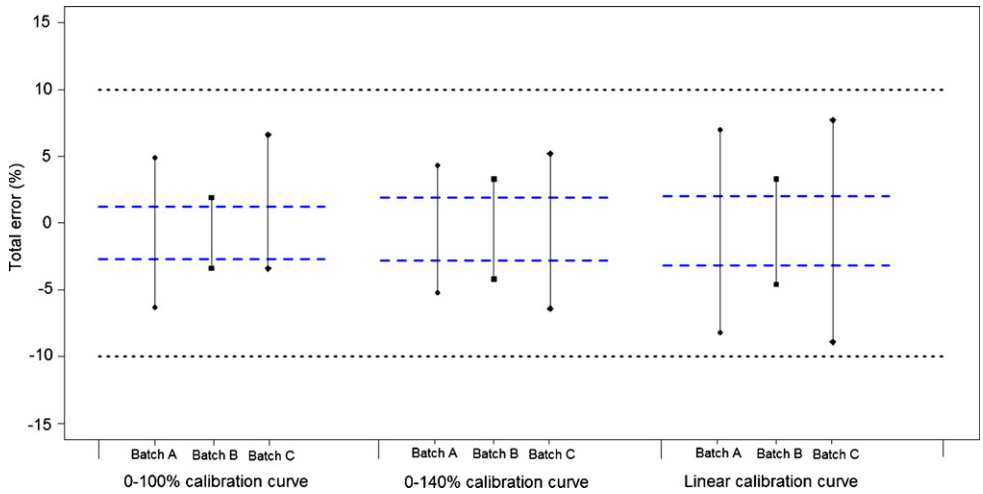

Fig. 4. Accuracy of the results obtained for the HPLC-UV method for the determination of methyl parahydroxybenzoate for the authentic batch samples (syrups) with the simple linear regression model, with the linear model forced through the origin and fitted only with the 100.0% calibration level or fitted only with the 140.0% calibration level. The vertical continuous segment are the 95% β-expectation tolerance intervals obtained with the authentic batch samples for each of the three batches, the dashed lines are the 95% β-expectation tolerance limits obtained with the 100% concentration level of the spiked validation standards and the dotted curves represent the acceptance limits (±10%).

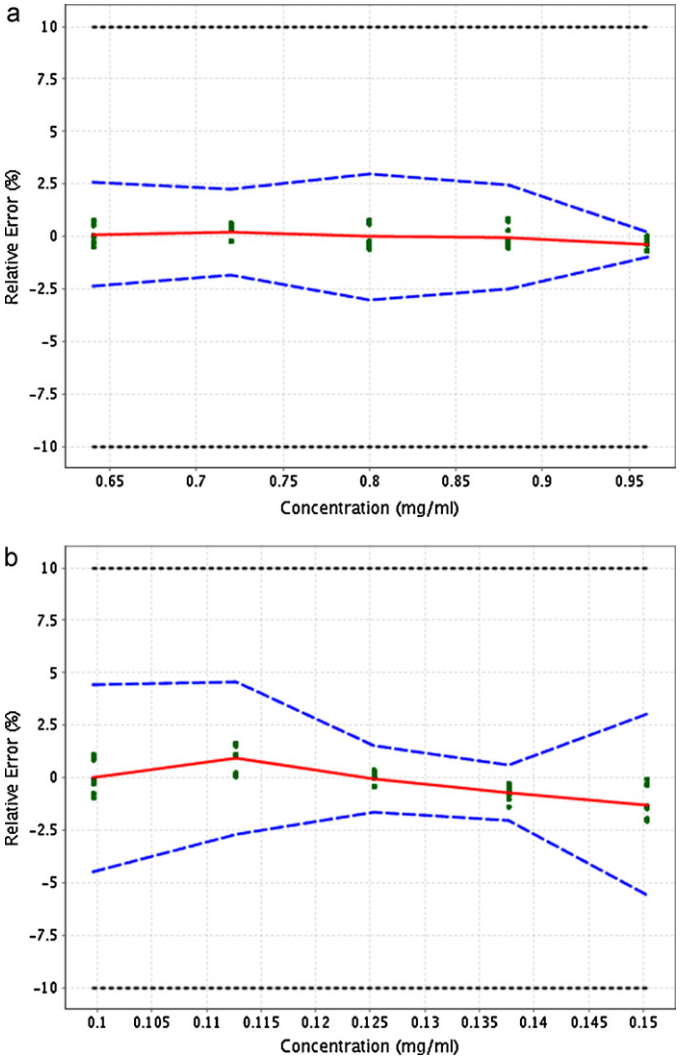

Fig. 5. Accuracy profiles of the HPLC-UV method for the simultaneous determination of (a) valsartan and (b) hydrochlorothiazide obtained for the spiked validation standards with the linear model forced through the origin and fitted only with the 120.0% calibration level. The plain line is the relative bias, the dashed lines are the 95% β-expectation tolerance limits and the dotted curves represent the acceptance limits (±10%). The dots represent the relative back-calculated concentrations of the validation standards.

thus confirming that, for these examples, the validation standards mimicked adequately the authentic batch samples.

None of the case studies depicted in this work showed the situation where the analysis of authentic batch impairs the conclusion of validity obtained with the validation standards. In such a situation, the regulatory guideline ICHQ2R1 [1] is the rule and should be followed. As this document requires to validate the method over a concentration range (e.g. 80–120% for the active substance in a drug product), that is only achievable using validation standards, the validity of the method should be defined by the analysis of those placebo based validation standards. Similarly the selection of the adequate standard curve should be defined when using validation standards. The use of authentic batch samples is here seen as a valuable confirmation of the method validity.

However, when the analysis of the authentic batch samples does not confirm the validity of the method, investigation of the origins of this failure should be realised as it leaves doubts about the usefulness of the method. Sources of these differences may arise from the sample preparation of the authentic batch samples. Indeed, this step is not necessarily present or similar when using placebo based validation standards. For instance some sources of additional variability may come from the segregation of electro-

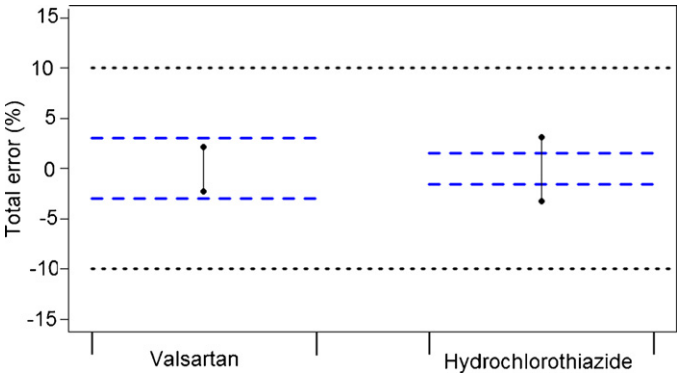

Fig. 6. Accuracy of the results obtained for HPLC-UV method for the simultaneous determination of valsartan and hydrochlorothiazide for the authentic batch samples (tablets) with the linear model forced through the origin and fitted only with the 120.0% calibration level. The vertical continuous segments are the 95% β-expectation tolerance intervals obtained with the authentic batch samples, the dashed lines are the 95% β-expectation tolerance limits obtained with the 100% concentration level of the spiked validation standards and the dotted curves represent the acceptance limits (±10%).

Table 4
p-Values obtained for the test of differences in variances obtained with the placebo spiked validation standards versus the authentic batch samples for each analyte studied: metformin, methyl parahydroxybenzoate, valsartan and hydrochlorothiazide.

Analyte	Method	Calibration curve	Batch no	*p*-Value
Metformin	Spectrophotometric-UV	Linear 0–100%	1	0.404
Methyl parahydroxybenzoate	HPLC-UV	Linear 0–140%	1	0.729
			2	0.712
			3	0.232
Valsartan Hydrochlorothiazide	HPLC-UV	Linear 0–120%	1	0.365
				0.152

static analytes when vigorously crushing tablets, from variability of the dissolution of the authentic samples, from the additional steps of filtration or even from inadequate homogenisation of the initial sample. Another additional source of variability could come from the matrix of the placebo based validation standards that may not match perfectly the one of the authentic batch samples.

The aim of adding to the method validation the analysis of authentic batch samples is to increase the confidence the analysts, laboratory, patients as well as regulatory bodies can have in the results generated by the analytical method under study.

5. Conclusions

Through the three examples presented in this work, the use of authentic batch samples (tablets or syrups) has been shown to valuably increase the reliability of the decision to declare an analytical method valid using only spiked placebo validation standards. Using authentic batch samples for the evaluation of results accuracy has been shown feasible. This study further illustrated that, depending on the type of response function used as calibration curve, there were various degrees of difference in the methods performance between the two types of samples: spiked validation standards and authentic batch samples. Thus adding these last samples into the validation design may help the analyst to select the most fit for purpose calibration curve and thus increase the reliability of the results generated by the method in routine application. Finally, using reconstituted validation standards seems a coherent practice for analytical methods validation as it has been shown in this work that they mimicked relatively well the behaviour of authentic batch samples. Although this last statement may not be applicable to all situations, the inclusion of authentic batch samples in any analytical method validation step would efficiently help analyst to answer the question about the transferability of the validity of a method from spiked validation standards to authentic batch samples.

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