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Flexibility and applicability of β-expectation tolerance interval approach to assess the fitness of purpose of pharmaceutical analytical methods

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An innovative versatile strategy using Total Error has been proposed to decide about the method's validity that controls the risk of accepting an unsuitable assay together with the ability to predict the reliability of future results. This strategy is based on the simultaneous combination of systematic (bias) and random (imprecision) error of analytical methods. Using validation standards, both types of error are combined through the use of a prediction interval or β-expectation tolerance interval. Finally, an accuracy profile is built by connecting, on one hand all the upper tolerance limits, and on the other hand all the lower tolerance limits. This profile combined with pre-specified acceptance limits allows the evaluation of the validity of any quantitative analytical method and thus their fitness for their intended purpose.

In this work, the approach of accuracy profile was evaluated on several types of analytical methods encountered in the pharmaceutical industrial field and also covering different pharmaceutical matrices. The four studied examples depicted the flexibility and applicability of this approach for different matrices ranging from tablets to syrups, different techniques such as liquid chromatography, or UV spectrophotometry, and for different categories of assays commonly encountered in the pharmaceutical industry i.e. content assays, dissolution assays, and quantitative impurity assays. The accuracy profile approach assesses the fitness of purpose of these methods for their future routine application. It also allows the selection of the most suitable calibration curve, the adequate evaluation of a potential matrix effect and propose efficient solution and the correct definition of the limits of quantification of the studied analytical procedures.

Keywords: accuracy profile; tolerance intervals; total error; analytical method validation

Introduction

In the pharmaceutical industry, efficient use of any analytical procedures requires to certainty that every future measurement in routine analysis, stability studies or process validation studies, and so on, will be close enough to the unknown true value of the amount or concentration of the analyte contained in the sample.

Classical approaches to validation only check quantitative performances against reference values, but this does not reflect the needs of the end users of the results generated by the analytical method.[1] An innovative versatile strategy using accuracy profile computed by means of β-expectation tolerance interval and total measurement error has been proposed to decide about the method's validity that controls the risk of accepting an unsuitable method together with the ability to predict the reliability of future results.

The concept of accuracy profile was first introduced in the papers of Hubert *et al.*[1] and Boulanger *et al.*[2] After that, in 2003, the commission on the validation of analytical procedures of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) used the accuracy profiles for improving the assessment of results' accuracy in method validation studies.[3]

Since then, several publications (over 70) have used this new approach in analytical validation,[1–78] 18 publications are devoted to discussions on the concept of this approach,[1–18] 31 have applied the approach in the validation of bioanalytical methods,[19–49] 10 for pharmaceutical methods,[50–59] 7 for the analysis of plant materials,[60–66] 6 to transfer of analytical methods from one laboratory to another,[67–72] and 6 publications for other applications (food processing, environmental control, etc.).[73–78]

The aim of this publication is to demonstrate the high applicability of this validation methodology in the pharmaceutical

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industry field and to illustrate its flexibility with regard to several types of pharmaceutical analytical methods.

To achieve this aim, this harmonized validation approach was applied to various analytical techniques (liquid chromatography (LC), UV spectrophotometry), for different types of matrices (tablet, syrup) and for different category of test (assay, dissolution, impurities). Each example was chosen because it illustrates a specific situation classically faced by analysts in the pharmaceutical industry.

All examples are presented following the same structure. A brief reminder of the procedure provides the type of analytical technique used, as well as the goals to be achieved. Then, the applied experimental design is presented with the description of the calibration and validation standards used. After that, the accuracy profiles obtained with the adequate response function model (the relationship between the response (signal) and the concentration (quantity) of the analyte in the sample, also sometime called calibration or standard curve) is presented and allows interpreting and deciding about the validity of the method. Finally, the corresponding trueness, precision, accuracy data, limit of quantification (LOQ), valid concentration range, as well as the higher and lower tolerance interval limits are then summarized in tables.

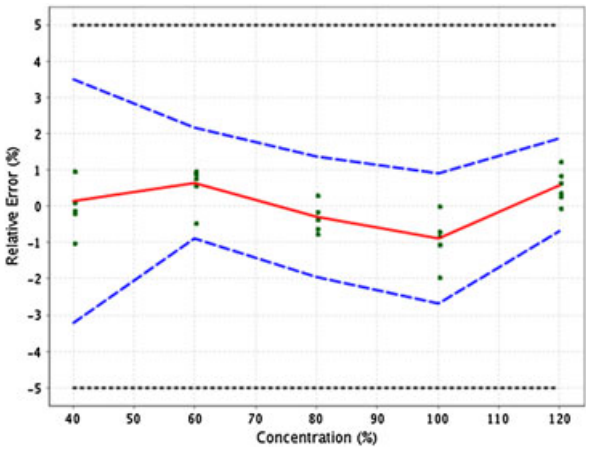

Figure 2. Accuracy profile of metformin determination in tablets by UV spectrophotometry obtained after application of linear regression using calibration standards prepared with the matrix. The plain line is the relative bias, the dashed lines are the 95% β-expectation tolerance limits and the dotted curves represent the acceptance limits (±5%). The dots represent the relative back-calculated concentrations of the validation standards.

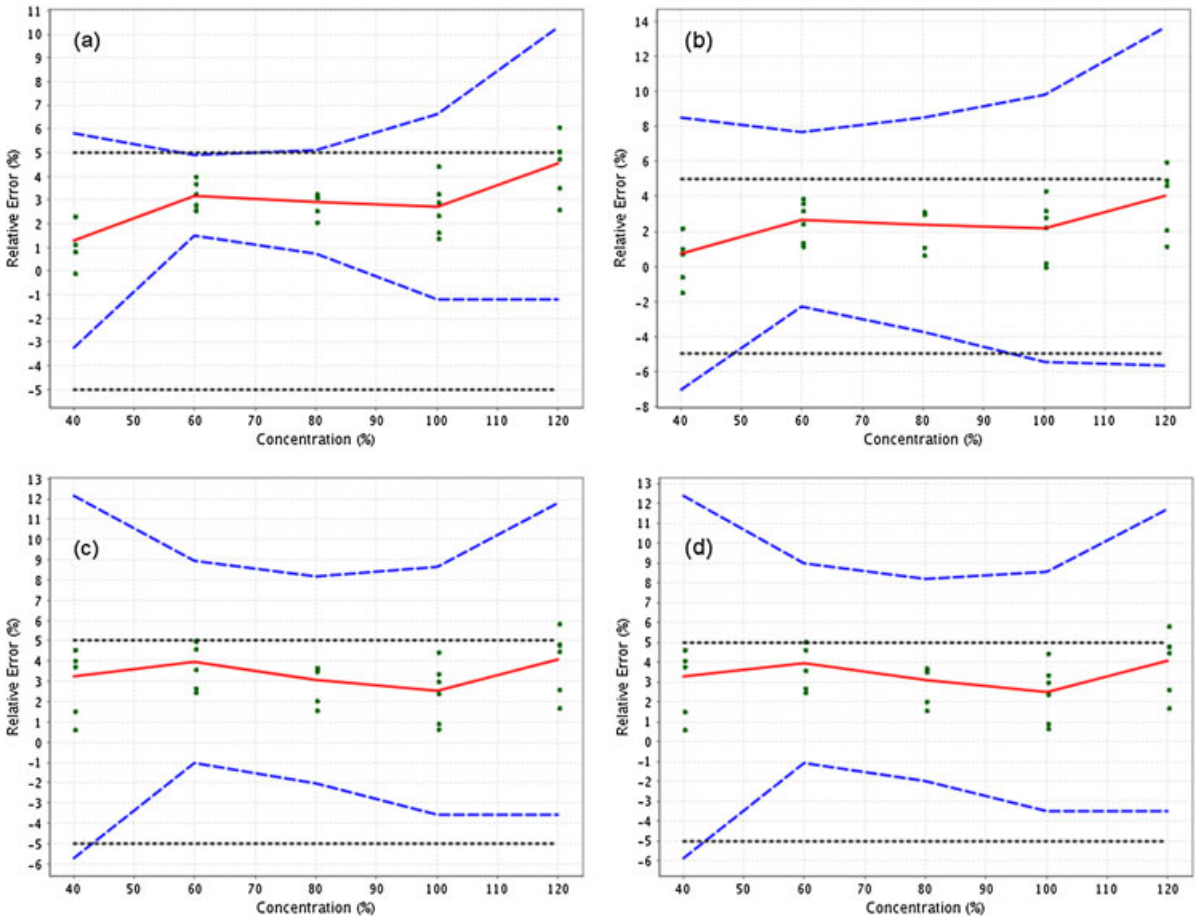

Figure 1. Accuracy profiles of metformin determination in tablets by UV spectrophotometry obtained using calibration standards prepared without the matrix after application of (a) Linear regression forced through the origin 0 fitted only with 100% calibration level; (b) Linear regression forced through the origin 0 fitted only with the highest calibration level; (c) Simple linear regression; and (d) Weighted (1/X) linear regression. 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 (±5%). The dots represent the relative back-calculated concentrations of the validation standards.

Table 1. Performance criteria of metformine content validation obtained with the model linear regression (a) within matrix and (b) without matrix

(a)		Linear regression within matrix		
Response function				
Y = a + bX		Run 1	Run 2	Run 3
	Intercept	−0.01693	−0.01619	0.008182
	Slope b	0.008663	0.008619	0.008337
Trueness				
		Absolute bias, % *w/w* (Relative, %)		
40 %		0.05705 (0.1)		
60 %		0.3824 (0.6)		
80 %		−0.2381 (−0.3)		
100 %		−0.8983 (−0.9)		
120 %		0.6969 (0.6)		
Precision				
		Repeatability (RSD, %)		Intermediate precision (RSD, %)
40 %		0.4		0.8
60 %		0.5		0.5
80 %		0.2		0.4
100 %		0.6		0.6
120 %		0.4		0.4
Accuracy				
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)		
40 %		[38.7 , 41.4] ; [−3.2 , 3.5]		
60 %		[59.5 , **61.3] ; [−0.9 , 2.2]**		
80 %		**[78.4 , 81.1] ; [−1.9 , 1.4]**		
100 %		**[97.3 , 100.9] ; [−2.7 , 0.9]**		
120 %		[119.2 , 122.3] ; [−0.7 , 1.8]		
Linearity				
		Interval (% *w/w*)		40 – 120
		Slope		1.000
		Intercept		−0.00008064
		r^2		0.9994
LOQ (lower)				40% (*w/w*)
(b)		Linear regression without matrix		
Response function				
Y = a + bX		Run 1	Run 2	Run 3
	Intercept	−0.01212	−0.01530	0.007683
	Slope b	0.008245	0.008269	0.008187
Trueness				
		Absolute bias, % *w/w* (Relative, %)		
40 %		1.295 (3.2)		
60 %		2.370 (4.0)		
80 %		2.466 (3.1)		
100 %		2.527 (2.5)		
120 %		4.926 (4.1)		
Precision				
		Repeatability (RSD, %)		Intermediate precision (RSD, %)
40 %		0.4		1.9
60 %		0.6		1.2
80 %		0.2		1.1
100 %		0.9		1.6
120 %		0.6		1.7
Accuracy				
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)		
40 %		[37.7 , 44.9] ; [−5.7 , 12.2]		
60 %		[59.4 , 65.4] ; [−1.0 , 8.9]		
80 %		[78.4 , 86.6] ; [−2.0 , 8.2]		

(Continues)

Table 1. *(Continued)*

(a)		Linear regression within matrix		
Response function				
Y = a + bX		Run 1	Run 2	Run 3
	Intercept	−0.01693	−0.01619	0.008182
	Slope b	0.008663	0.008619	0.008337
100 %		[96.4 , 108.6] ; [−3.6 , 8.6]		
120 %		[115.7 , 134.2] ; [−3.6 , 11.8]		
Linearity				
	Interval (% *w/w*)			40 – 120
	Slope			1.037
	Intercept			−0.2505
	r^2			0.9982
LOQ (lower)				No LOQ

Materials and methods

Determination of metformin in tablets by UV spectrophotometry

Chemicals and reagents

Metformin hydrochloride standard reference was supplied by the European Pharmacopoeia (Strasbourg, France).

Instrumentation

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.

Calibration and validation standards

The following samples were prepared:

- **Calibration standards**: Prepared out of the matrix, 3 concentration levels: 80, 100, 120%, 2 repetitions per level during 3 days. The same calibration standards are prepared with the matrix.
- **Validation standards**: Prepared in a reconstituted matrix (placebo) at 3 concentration levels: 80, 100, 120% of the target value, analyzed during 3 days. The levels 80 and 120% were replicated twice per day of analysis, while for the level 100% it was 6 times.

Determination of fluconazole in dissolution media by high performance liquid chromatography (HPLC)

Chemicals and reagents

All chemicals and solvents used were of analytical or HPLC grade. Fluconazole was supplied by the European Pharmacopoeia. Potassium dihydrogen phosphate was purchased from Acros Organics (Geel, Belgium).

Methanol and phosphoric acid (85%) were obtained from Merck (Darmstadt, Germany). Deionized water was generated from the Milli-Q water purifying system (Millipore, Watford, UK).

Instrumentation and chromatographic conditions

Analyses were performed on an Agilent technologies HPLC 1100 series system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a solvent delivery quaternary pump G1311A, an on-line degasser G1322A, an autosampler G1313A, a column oven G1316A and a diode-array detector G1315A.

Chromatographic analysis was performed on a C18 Thermo Hypersil BDS column (150 x 4.6 mm i.d., 5 μm particle size) and kept at 25 °C. The mobile phase was prepared by mixing separately measured methanol and phosphate aqueous solution (prepared by dissolving 1.4 g of potassium dihydrogen phosphate in 1000 ml of water and adjusted to pH 3.6 with phosphoric acid) in a ratio 40:60 (*v/v*) and was degassed before use. The HPLC system was operated isocratically at a flow rate of 1.0 ml/min and the injection volume was 20 μl. UV detection was performed at 261 nm.

Calibration and validation standards

A stock solution of fluconazole was prepared by accurately weighting 100.0 mg of fluconazole and diluting this in 100.0 ml of methanol. The calibration standards for fluconazole were prepared by diluting an appropriate volume of stock solution with 0.1 M HCl aqueous solution to reach the concentration levels corresponding to the range 25% to 125% of fluconalzole i.e. 12.5 (25%), 42.5 (85%), 50 (100%), 57.5 (115%) and 62.5 mg (125%) of fluconazole. The standards were analyzed in duplicate.

The validation standards were prepared similarly with addition of the excipients of the formulation of the drug product to reach four concentration levels ranging from 25% to 125% of fluconazole i.e. 12.5 (25%), 25.52 (50%), 50 (100%), and 62.5 mg (125%) of fluconazole. Each solution of the validation standard is introduced in two breakers and each validation standard (from each breaker) was analyzed two times. So the validation study was performed on six different series leading to a total of 96 analyses.

Determination of methyl parahydroxybenzoate content (preservative) in syrup by HPLC

Chemicals and reagents

Methyl parahydroxybenzoate standard reference was supplied by the European Pharmacopoeia. Acetonitrile of HPLC grade was purchased from Merck. Sodium acetate of analytical grade was supplied from Sigma-Aldrich (Steinheim, Germany).

Deionized water was generated from a Milli-Q water purifying system.

Instrumentation and chromatographic conditions

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 (1.36 g of sodium acetate in 1000 ml water adjusted to pH 5.0 with glacial acetic acid). The chromatographic isocratic separation was made with a Nucleosil C18 column (150 x 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.

Calibration and validation standards

Calibration standards and validation standards were prepared at five concentration levels: 60% (9 μg/ml), 80% (12 μg/ml), 100% (15 μg/ml), 120% (18 μg/ml) and 140% (21 μg/ml) of the target nominal concentration of methyl parahydoxybenzoate in the syrup formulation. The calibration standards prepared in methanol were analyzed once during 3 days. Each validation standard prepared in a reconstituted matrix of the formulation (or placebo) was analyzed in triplicates during the same three days, except the 100% level that was replicated 6 times each day.

Quantification of detection of known impurities in amoxicilline tablets

Chemicals and reagents

Amoxicilline standard reference was supplied by the European Pharmacopoeia. Acetonitrile of HPLC Grade was purchased from Merck. KH2PO4of analytical grade was supplied from Sigma-Aldrich. Deionized water was generated from a Milli-Q water purifying system.

Instrumentation and chromatographic conditions

The HPLC system consisted is the same LaChrom system previously described. The mobile phase consisted in a 96/4 (*v/v*) mixture of acetonitrile and an aqueous buffer (6.8 g of KH_2PO_4 in 1000 ml of water, adjusted to pH 5.0 with KOH 45% (*m/v*)). The chromatographic isocratic separation was made with a Lichrosphere C18 column (250 x 4.6 mm ID; particle size: 5 μm) and the UV detection was performed at 230 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.

Calibration and validation standards

Calibration standards were prepared at twelve concentration levels: 20% (5 mg), 40% (10 mg), 50% (12.5 mg), 60% (15 mg), 80% (20 mg), 90% (22.5 mg), 100% (25 mg), 110% (27.5 mg), 120% (30 mg), 140% (35 mg), 150% (37.5 mg), and 160% (40 mg) of the specification of three impurities (impurity A, B & C). The calibration standards prepared in the mobile phase were analyzed twice during 3 days. Each validation standard was prepared in a reconstituted matrix of the formulation (or placebo) at five concentration levels (80%, 90%, 100%, 110%, and 120%) and was analyzed in duplicates during the same three days.

Results and discussion

Determination of metformin in tablets by UV spectrophotometry

By using the approach proposed by Hubert *et al.*, [6,11,12] the method is considered as not valid within the studied range of concentration when the accuracy profile crosses the accuracy acceptance limits set at ± 5%. This validation 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%.

Four calibration models were investigated, namely the simple linear regression, the linear regression forced through the origin (0) fitted only with the 100% calibration level, the linear regression

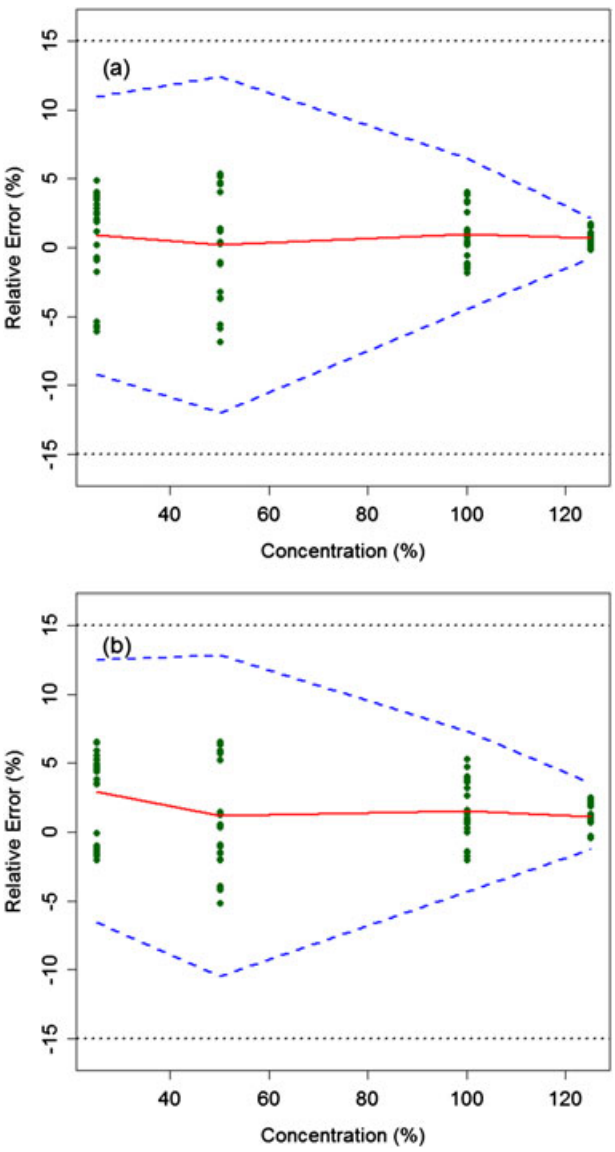

Figure 3. Accuracy Profiles of fluconazole dissolution obtained after application of linear regression through 0 fitted using the highest level only (a) and simple linear regression (b) The plain line is the relative bias, the dashed lines are the 95% β -expectation tolerance limits and the dotted curves represent the acceptance limits (±15%). The dots represent the relative back-calculated concentrations of the validation standards.

Table 2. Performance criteria of fluconazole dissolution obtained with the model; linear Regression through 0 fitted using the highest level only (a) and simple linear regression (b)

a) linear regression through 0 fitted using the highest level only

Response function							
Y = b x		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
	Slope b	1.2276E + 04	1.2276E + 04	1.2349E + 04	1.2349E + 04	1.2262E + 04	1.2262E + 04
Trueness							
		Absolute bias, % *w/w* (Relative, %)					
25 %		0.2240 (0.9)					
50.%		0.0870 (0.2)					
100.%		1.034 (0.9)					
125.%		0.8782 (0.7)					
Precision							
		Repeatability (RSD, %)			Intermediate precision (RSD, %)		
25 %		0.5			3.7		
50.%		0.4			4.4		
100.%		0.6			2.0		
125.%		0.2			0.5		
Accuracy							
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)					
25 %		[23.0 , 28.2] ; [−9.2 , 10.9]					
50.%		[44.8 , 57.4] ; [−12.1 , 12.4]					
100.%		[96.4 , 107.6] ; [−4.5 , 6.5]					
125.%		[122.8 , 127.2] ; [−0.7 , 2.1]					
m							
		Interval (%, *w/w*)			25 – 125		
		Slope			1.009		
		Intercept			−0.06430		
		r^2			0.9985		
LOD					1.45 (%, *w/w*)		
LOQ					Lower LOQ (%, *w/w*) = 25		
					Upper LOQ (%, *w/w*) = 125		

b) Linear regression

Response function							
Y = a + b x		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
	Slope b	1.2472E + 04	1.2472E + 04	1.2290E + 04	1.2290E + 04	1.2130E + 04	1.2130E + 04
	Intercept	−8803	−8800	1187	1190	−2028	−2030
Trueness							
		Absolute bias, % *w/w* (Relative, %)					
25 %		0.7446 (2.9)					
50.%		0.5956 (1.2)					
100.%		1.5146 (1.5)					
125.% ,		1.3972 (1.1)					
Precision							
		Repeatability (RSD, %)			Intermediate precision (RSD, %)		
25 %		0.5			3.5		
50.%		0.4			4.2		
100.%		0.6			2.2		
125.%		0.2			0.9		
Accuracy							
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)					
25 %		[23.6 , 28.4] ; [−6.6 , 12.5]					
50.%		[45.6 , 57.6] ; [−10.4 , 12.8]					
100.%		[96.6 , 108.4] ; [−4.3 , 7.3]					
125.%		[123.0 , 129.0] ; [−1.3 , 3.5]					
Linearity							
		Interval (%, *w/w*)			25 – 125		
		Slope			1.009		

(Continues)

Table 2. *(Continued)*

a) linear regression through 0 fitted using the highest level only

Response function							
Y = b x		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
	Slope b	1.2276E + 04	1.2276E + 04	1.2349E + 04	1.2349E + 04	1.2262E + 04	1.2262E + 04
	Intercept				0.1933		
	r^2				0.9984		
LOD					5.27 %, *w/w*		
LOQ					Lower LOQ (%, *w/w*) = 25		
					Upper LOQ (% *w/w*) = 125		

forced through the origin (0) fitted only with the 120% calibration level and a weighted (1/X) linear regression. Accuracy profiles obtained with these calibration curves are shown in Figure 1.

As illustrated in Figure 1, the method is thus considered not valid over the concentration range investigated whatever the calibration model used. It can be observed that the tolerance intervals are outside the ± 5% acceptance limits.

In order to understand the problem encountered with this validation, we suspected the presence of matrix effect problem. This was observed by using calibration standards prepared with all the excipients to construct different response functions. Indeed, after using these calibration curves prepared within the matrix, the method can now be considered as valid. This is shown on Figure 2 that depicts the accuracy profile obtained after application of linear regression with calibration standards prepared within the matrix.

Indeed, with the classical validation methodologies, [15,2,10,78–84] when a matrix effect is detected, the usually decision made is to go back to the development or optimization phase of the method or simply its rejection. So this example illustrates that an analytical method can still be useful and valid with this new validation approach, even if a matrix effect has been detected. A solution to circumvent this matrix effect problem is to prepare calibration standards with all the matrix components.

Table 1 presents the validation criteria such as trueness, precision, accuracy of the results, linearity of the results, and the limit of quantification obtained with the linear calibration curve prepared with all the matrix components included into the calibration standards (Table 1a) as well as the validation criteria obtained with the linear calibration curve prepared without the matrix component (Table 1b) for comparison. As can be seen in Table 1b, the main problem arising from using a calibration curve prepared without the matrix component is that it provides a high relative bias (ranging from 2.5 to 4.1%) while intermediate precision RSD never exceeds 2.0%. As it seems that the good precision of the method could compensate the weakness of the trueness of the method, it could be tempting to declare this method as valid. However the accuracy profile depicted in Figure 1c and the values of the relative 95% β-expectation tolerance intervals show that there is high risk that the method will provide inaccurate and unreliable results when performed with calibration standards prepared without the matrix. Indeed, from the various methodologies available to decide about the validity of an analytical method, the accuracy profile approach using statistical tolerance is almost the only one providing that the results will be of enough reliability.[15] This example of

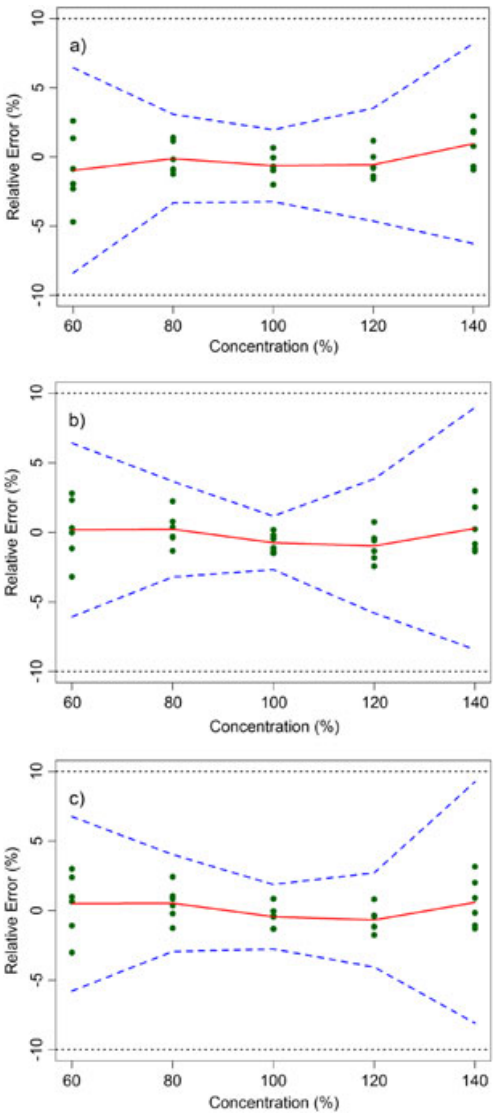

Figure 4. Accuracy profiles of the HPLC-UV method for the determination of methyl parahydroxybenzoate obtained for the validation standards with the simple linear regression model (a) and with the linear model forced through the origin and fitted only with the 100.0% calibration level (b) or fitted only 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.

analytical method validation aiming at controlling the concentration of an active ingredient in tablets illustrates the accuracy profile approach's flexibility in regards to matrix effects. It allows demonstrating that a method can be validated even if a matrix effect is present. It further guarantees the quality of the future results that will be generated by the validated analytical method.

Determination of fluconazole in dissolution media by HPLC

In order to evaluate the validation of the analytical method aiming at quantifying fluconazole in dissolution baths with the total error approach, we have to set the acceptance limits. Such limits are missing in the guidelines. We will compare the dissolution test to the test of uniformity of content of single-dose preparations.

In the European Pharmacopoeia[85] definition of the uniformity content test we found that: *the test . . . is based on the assay of the individual contents of active substance(s) of a number of single-dose units to determine whether the individual contents are within limits set with reference to the average content of the sample*.

This definition is similar to the definition of dissolution tests as mentioned in the European Pharmacopoeias since the objective of a dissolution test is the assay of the individual contents of active substance(s) of each breaker in order to determine whether the individual contents are within limits set with reference to the average content of the sample. In another paragraph of the same document,[85] we found that these limits are set at 85% and 115% of the nominal concentration. Therefore, the acceptance limits are set at +/−15% for the analytical method validation and the risk is set at 5%.

Several calibration models were tested to analyze the relationship between the amount of fluconazole (in mg) and the instrumental response (the chromatographic peak area) and decide which model is most appropriate. Two models were relevant to perform the calibration and back calculate the concentrations of the validation standards: the simple linear regression and the regression through the origin fitted to the 62.5 mg (125%) calibration standard. The obtained accuracy profiles are presented in Figure 3. Table 2 presents the method's validation criteria obtained with these models. As shown in Figure 3 and Table 2, the method of dissolution of fluconazole is valid with these two

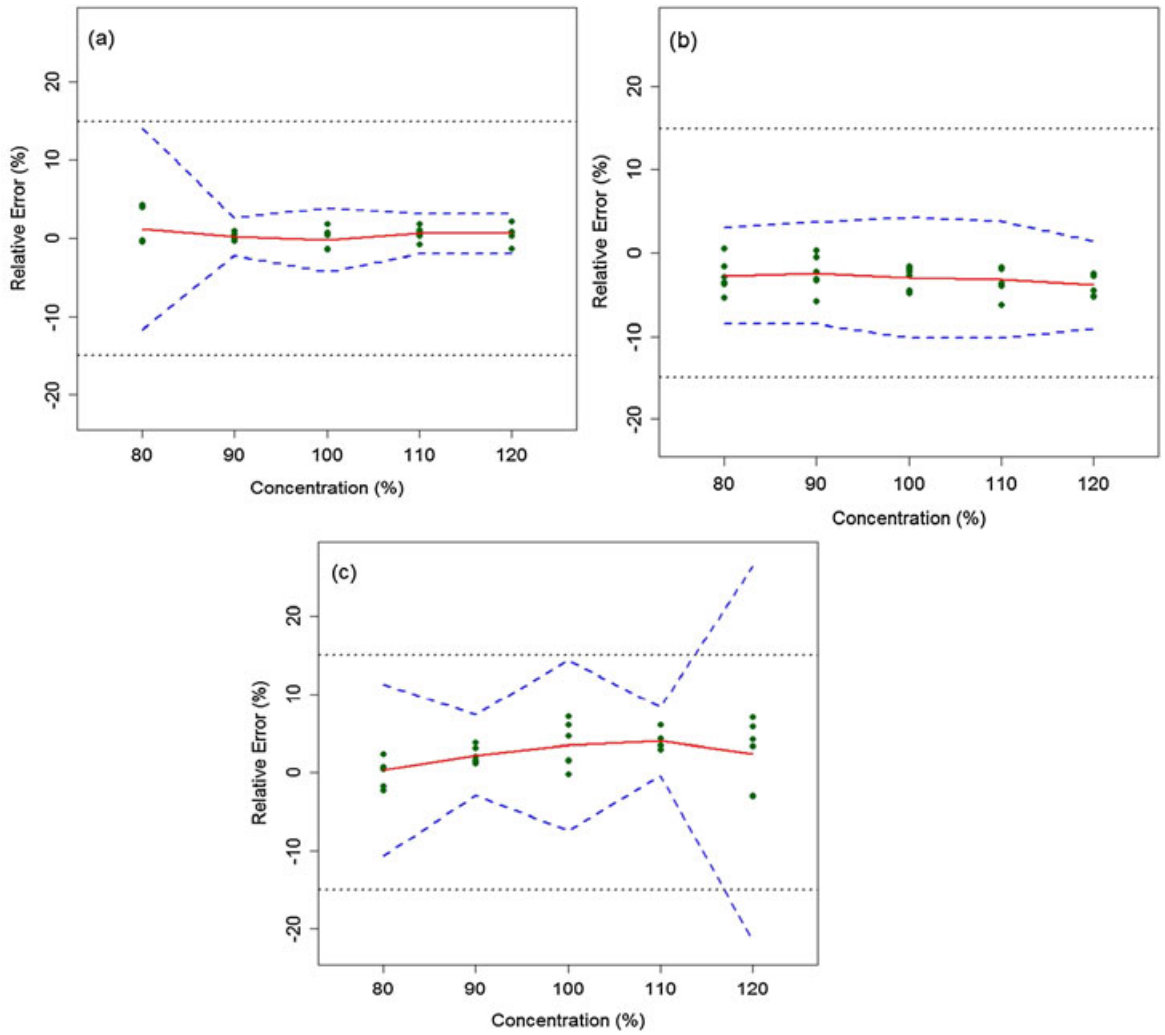

Figure 5. Accuracy profiles obtained after application of a linear regression model forced through the origin and fitted only with the highest level of the calibration standards for Impurity A (a), Impurity C (b), and Impurity D (c). The plain line is the relative bias, the dashed lines are the 95% β-expectation tolerance limits and the dotted curves represent the acceptance limits (±15%). The dots represent the relative back-calculated concentrations of the validation standards.

Table 3. Performance criteria of method validation obtained with a linear regression model forced through the origin and fitted only with the highest level of the calibration standards for Impurity A (a), Impurity C (b), and Impurity D (c)

a) Impurity A

Response function				
Y = b x		Run 1	Run 2	Run 3
	Slope b	4095	4164	4395
Trueness				
		Absolute bias, %, *w/w* (Relative, %)		
80 %		0.96 (1.2)		
90%		0.18 (0.2)		
100%		−0.20 (−0.2)		
110 %		0.66 (0.6)		
120%		0.72 (0.6)		
Precision				
		Repeatability (RSD, %)		Intermediate precision (RSD, %)
80 %		0.2		2.6
90%		0.3		0.6
100%		1.3		1.4
110 %		0.8		0.9
120%		0.9		1.2
Accuracy				
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)		
80 %		[70.64 , 91.28] ; [−11.7 , 14.1]		
90%		[88.02 , 92.34] ; [−2.2 , 2.6]		
100%		[95.80 , 103.8] ; [−4.2 , 3.8]		
110 %		[107.91 , 113.52] ; [−1.9 , 3.2]		
120%		[116.04 , 125.40] ; [−3.3 , 4.5]		
Linearity				
		Interval (%, *w/w*)		80 – 120
		Slope		0.9999
		Intercept		0.1194
		r^2		0.9922
LOD				0.49 %, *w/w*
LOQ				Lower LOQ (%, *w/w*) = **80**
				Upper LOQ (%, *w/w*) = 120

b) Impurity C

Response function				
Y = b x		Run 1	Run 2	Run 3
	Slope b	2211	2272	2464
Trueness				
		Absolute bias, %, *w/w* (Relative, %)		
80 %		−2.16 (−2.7)		
90%		−2.16 (−2.4)		
100%		−2.90 (−2.9)		
110 %		−3.41 (−3.1)		
120%		−4.56 (−3.8)		
Precision				
		Repeatability (RSD, %)		Intermediate precision (RSD, %)
80 %		2.0		2.0
90%		2.1		2.2
100%		0.4		1.5
110 %		1.2		1.9
120%		0.8		1.4
Accuracy				
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)		
80 %		[73.20 , 82.40] ;[−8.5 , 3.0]		
90%		[82.35 , 93.33] ;[−8.5 , 3.7]		

(Continues)

Table 3. *(Continued)*

a) Impurity A

Response function				
Y = b x		Run 1	Run 2	Run 3
	Slope b	4095	4164	4395
100%		[89.90 , 104.20] ;[−10.1 , 4.2]		
110 %		[98.89 , 114.18] ;[−10.1 , 3.8]		
120%		[109.2 , 121.68] ;[−9.0 , 1.4]		
Linearity				
	Interval (%, *w/w*)		80% – 120%	
	Slope		0.9393	
	Intercept		0.7538	
	r^2		0.9851	
LOD			3.47 %, *w/w*	
LOQ			Lower LOQ (%, *w/w*) = 80	
			Upper LOQ (%, *w/w*) = 120	

b) Impurity D

Response function				
Y = b x		Run 1	Run 2	Run 3
	Slope b	1.0629E + 04	1.0893E + 04	1.1004E + 04
Trueness				
		Absolute bias, %, *w/w* (Relative, %)		
80 %		0.24 (0.3)		
90%		1.98 (2.2)		
100%		3.49 (3.5)		
110 %		4.29 (3.9)		
120%		2.88 (2.4)		
Precision				
		Repeatability (RSD, %)		Intermediate precision (RSD, %)
80 %		0.3		2.2
90%		0.3		1.1
100%		2.0		3.1
110 %		0.7		1.2
120%		0.6		4.9
Accuracy				
		β-expectation tolerance limit (% *w/w*) ; relative β-expectation tolerance limit (%)		
80 %		[71.44 , 89.04] ;[−10.7 , 11.3]		
90%		[87.30 , 96.66] ;[−3.0 , 7.4]		
100%		[92.60 , 114.40] ;[−7.4 , 14.4]		
110 %		[109.56 , 119.24] ;[−0.4 , 8.4]		
120%		[94.20 , 151.68] ; [−21.5 , 26.4]		
Linearity				
	Interval (%, *w/w*)		80% – 110%	
	Slope		1.080	
	Intercept		−1.368	
	r^2		0.9597 .	
LOD			4.02%, *w/w*	
LOQ			Lower LOQ (%, *w/w*) = **80**	
			Upper LOQ (%, *w/w*) = 110	

calibration models: simple linear regression and regression through the origin fitted to the 62.5 mg (125%). As in both cases it is guaranteed that each future results will be within the +/−15% acceptance limits, the analyst is free to select the most simple one. In this case the calibration model that would finally be selected would be the one using the regression through the origin fitted to the 62.5 mg (125%) as it is the most efficient situation.

With this example, we demonstrated:

- The applicability of the accuracy profile as an adequate validation approach for the dissolution test.
- The possibility to use new factors to set the series, others than the one usually used such as days, equipments or operators. For this dissolution test, a combination of two factors was selected: days (at three levels) and breaker (at two levels (as part

of equipment). As discussed by Rozet *et al*.,[10] this concept of series or runs which can take several dimensions must include the appropriate factors for the analytical method and in the case of dissolution tests breakers are important components of the equipments.
- The problem of lack of acceptance criteria in the guidelines for some tests has been discussed (here dissolution test) and acceptance limits for the dissolution tests have been proposed.

Determination of methyl parahydroxybenzoate content (preservative) in syrup by HPLC

The accuracy profile approach[6,11,12] 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 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 Figure 4.

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 simplest calibration model, it provides the least bias and leads to the least extrapolation of results.

With this example the applicability of the accuracy profile approach for the validation of an assay aiming at quantifying a preservative in a drug product was illustrated.

Quantification of known impurities in amoxicilline tablets

Several calibration models were tested to find the adequate response function. The linear regression model was suitable to perform the calibration and back calculate the concentrations of the validation standards as shown by the accuracy profile of Figure 5. Table 3 presents the validation criteria obtained for the analysis of the impurities. The acceptance limits were set at + / -15% and the risk at 5%.

Impurity A & C

The accuracy profiles obtained for impurity A and C (Figures 5a and 5b) show that the method is valid and allows to quantify these impurities with adequate reliability over the whole concentration range investigated. Indeed, these two profiles can ensure that each future result obtained for these two analytes will be within the acceptance limits of±15% with 95% probability.

Impurity D

The accuracy profile of impurity D (Figure 5c) shows that the tolerance interval of the highest level of the validation standard is not inside the ±15% acceptance limits. We can conclude that the method is only valid on the interval [80–110%].

This example highlights the fact that the β-expectation tolerance interval approach can be used successfully for the validation of quantitative impurity assays.

Conclusions

Through the different examples presented in this work, the use of the accuracy profile as a validation approach for different matrices and different techniques and for different categories of assays commonly encountered in the pharmaceutical industry has been shown. Its applicability as an efficient validation methodology in the pharmaceutical industry is demonstrated. The accuracy profile approach assesses the fitness for purpose of these methods for their future routine application.

With the accuracy profile approach, the most important in the validation is the intended objective of this method in routine and nothing else; this is based on comparing the predictive distribution of the results to the acceptance limits to decide about the validity of a method.

With these examples, we also demonstrated:

- The flexibility to consider the matrix effect and the possibility of any analytical method to be useful even with its matrix effect.
- The concept of series or runs must incorporate the appropriate factors for the analytical method and must take several dimensions in addition of the classical factors such day/ equipment / operator.

Moreover, we have underlined the problem of the absence in the guidelines of acceptance criteria for several tests: quantitative impurities tests, dissolution tests and determination of preservative content, etc. We have thus proposed several acceptance criteria for some of these tests.

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