ELSEVIER

Journal of Chromatography A, 815 (1998) 173–182

JOURNAL OF CHROMATOGRAPHY A

Methodology for transfer of liquid chromatography methods based on statistical considerations

Jérôme Vial[a], Alain Jardy[a,*], Pascal Anger[b], André Brun[b], Jean-Michel Menet[b]

[a]*Laboratoire ''Environnement et Chimie Analytique'', Ecole Supérieure de Physique et Chimie Industrielles de la Ville de Paris, 10 Rue Vauquelin, 75005 Paris, France*

[b]*Centre de Recherche de Vitry Alfortville, Rhône-Poulenc Rorer, 13 Quai Jules Guesde, 94400 Vitry sur Seine, France*

Received 2 March 1998; received in revised form 9 June 1998; accepted 9 June 1998

Abstract

An important task in the pharmaceutical industry today is analytical transfer. However, no actual guidelines are available today. It is for this reason that we decided to devise a rigorous method using statistic exploitation of results. The statistical technique used is ANOVA (analysis of variance). We chose to treat the case of quantitative analysis in LC but the methodology could easily be adapted to other analytical techniques. The criteria of the transfer validation could be formulated thus: ''for each response of interest, the new laboratory must produce results that are not significantly different from those of the reference entity'', or more explicitly by: ''the new laboratory must have dispersion characteristics compatible with those of the reference entity and must exhibit no bias''. While compatibility of precision can easily be assessed, the test of absence of bias requires that certified materials be available. Since certified materials can only be obtained through an inter-laboratory study, it means that the reference entity is necessarily a pool of laboratories. Using a single laboratory instead of a pool would not allow a distinction to be drawn between a bias and an inter-laboratory dispersion, which would lead to abnormal transfer failure. The methodology developed was then used on an example. The last part deals with the situation where certified materials are suspected to be slightly degraded. It is explained how such a case, likely to be encountered in pharmaceutical products stored over a long period, can be handled without re-starting the study from scratch.

Keywords: Pharmaceutical analysis; Statistical analysis; Analysis of variance; Method transfer

1. Introduction and preliminaries

Because of the complete automation of chromatographic devices, the repeatability of analyses has reached a high level of excellence (with R.S.D. less than 1%). But problems arose: when two laboratories analyzed quantitatively the same product with the same liquid chromatography (LC) method, significant differences appeared quite frequently, which formed the subject of many studies [1–14]. For the pharmaceutical industry, where regulation has tended to become more and more strict [15], this situation was a real problem. Henceforth industrial concerns had to validate any analytical transfer. The validation note must prove that the results of the new laboratory were not significantly different from the reference results. However, it was current practice that only two laboratories were involved in the study. Such an approach could be acceptable if the laboratory that had developed the method could assert that all the

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00510-X

parameters influent had been identified and could be controlled. It is quite seldom with a chromatographic method. So, in a two-laboratory transfer attempt, if significant discrepancies were observed it was impossible to determine whether the differences were tolerable and could be explained by usual inter-laboratory dispersion inherent to the method (and due to uncontrolled parameters), or reflected an unacceptable bias.

Current practices were also to define a priori validation criteria that had to be fulfilled. These criteria were determined by the analysts in charge of the method. In most cases they were based on instinct, on what was judged reasonable, and very often only on the results emanating from the laboratory that had developed the method, considered de facto as the reference entity.

This paper aims to give chromatographers a tool based on a rigorous method to validate a laboratory for the assay determination of a specified product. The statistical tool was not used with a view to giving the smallest confidence interval for a result or the smallest prediction interval. It must rather be seen as a means to establish objective criteria for validation of a new laboratory. This method is based on the use of the results of a collaborative study, which must necessarily have been completed previously. Here only the main principles governing such a study were recalled (for an exhaustive description refer previous works dealing with the spiramycin and ketoprofen studies [16,17]). Indeed, a laboratory was defined as an analyst–device pair, not as a geographical location. The number of laboratories involved was not fixed; however a minimum of six was required. These laboratories had to be representative of the population of the laboratories that could be induced to carry out such an analysis. In others words, their performance and way of performing the analysis were similar to those which conducted the method routinely. It was recommended to use as many batches as necessary to obtain a set able to be considered representative of the population of the potential batches. The injection sequence was not imposed a priori, but it must be identical for the collaborative study and the laboratory wishing to be validated. A sequence with three preparations per batch and two injections per preparation was recommended.

Subsequently, a statistical exploitation of the data had to be carried out. The usual technique employed was analysis of variance (ANOVA) [18]. The mathematical model required special care, since it had to be in accordance with the experimental design. This design generally included the factors laboratory, batch, preparation and the laboratory–batch interaction. The preparation factor is not included into the repeatability because it allows a better overview of the method characteristics and weaknesses. Moreover it does not require more experimental work and the additional computation work (easily achieved by a statistical software) is widely compensated by information obtained. Thanks to this information it is possible to improve the experimental protocol, for example through an optimization of the injection sequence. For each component of interest in the product, the conclusions of the collaborative trials must include not only the typical repeatability of the method characterized by its standard deviation (or variance) estimate, but also the influence or non-influence of the various factors or interactions. In the event of significant influence of a term, an estimation of the corresponding standard deviation (S.D.) was given. It was of interest to present these results in a table (Table 1).

A similar table must be set up for each component of interest in the product (e.g., likely to be used for

Table 1
Conclusions of the collaborative study

Source of variation	Significant influence (Y/N)	S.D. estimate	Degrees of freedom
Laboratory	?	$\hat{\sigma}_{lab}$	$n_{lab}-1$
Batch	?	$\hat{\sigma}_{batch}$	$n_{batch}-1$
Laboratory/batch interaction	?	$\hat{\sigma}_{lab/batch}$	$(n_{lab}-1)(n_{batch}-1)$
Preparation	?	$\hat{\sigma}_{prep}$	$n_{lab}n_{batch}(n_{prep}-1)$
Residual (repeatability)		$\hat{\sigma}_{r}$	$n_{lab}n_{batch}n_{prep}(n_{inj}-1)$

n_i stands for the number of modalities of factor i, (i$\in$ {laboratory, batch, preparation, injection}).

validation of a new laboratory in reference to the collaborative study).

In addition, the collaborative study enabled certified assays to be given for the batches analyzed. These batches could henceforth be used as reference materials for validation of a new laboratory. If significant influence of the interaction was exhibited it could signify insufficient resolution for some participants, or other problems of this kind. Further investigations concerning the origin of this problem must be undertaken in order to make appropriate corrections. Such an attitude would prevent future difficulties when transferring the method.

To illustrate and to facilitate understanding, an example was considered and the case where certified materials might be degraded is treated in Section 5.

2. Experimental conditions

This relates to a new laboratory wishing to be validated for the assay determination of some components of a product. It is worthy to note that a laboratory having taken part in the collaborative study and that the results of which have not been rejected as outliers is validated de facto.

The batches to be used are the certified assay batches of the collaborative study. The number can be reduced if it has been proven that some of these batches were non-informative for the kind of transfer chosen. The sample preparation technique must be identical to what would be done in routine analysis. If the collaborative study has been well-devised it must be very close to it, even identical. First, a new laboratory wishing to have itself validated must pass the conformity test, as each participant in the collaborative study has done previously. The injection sequence used in the new laboratory and in the collaborative study must be identical; however, if fewer batches are used, it may be curtailed.

3. Data processing

This treatment is to be applied to each component of the product whose assay determination must be validated by the new laboratory.

3.1. Calculation mode for assay from the proposed injection sequence

The assay calculation mode for the new laboratory must be the same as that used during the collaborative study. According to recommendations of the US Food and Drug Administration (FDA), for each injection of a product, it is advised that the assay is calculated using all the injections of the standard.

3.2. Statistical computations

The controlled factors that are taken into account are presented in Table 2.

The mathematical design is nested: the preparation factor is nested with the factor batch, which means the preparation i for batch j has nothing in common with preparation i for batch k.

According to this model, the theoretical expression for the αth result is:

$$x_{jk\alpha} = m + b_j + c_{k(j)} + \varepsilon_{jk\alpha} \quad (1)$$

where $x_{jk\alpha}$ is a result of a determination carried out on batch j with preparation $k(j)$, m is the expected value of x (estimated by the grand mean), b_j is the effect of the batch factor at level j, $c_{k(j)}$ is the effect of the preparation factor at level k, the factor batch being at level j, and $\varepsilon_{jk\alpha}$ is the effect of the random error.

The corresponding theoretical table for analysis of variance is shown in Table 3.

To calculate the ''sum of squares'' quantities, the use of statistical software is strongly recommended.

Again with the help of a software, for each component of interest in the product, it is possible to determine the repeatability variance of this new laboratory (estimated by $\hat{\sigma}_r^2$), the potential influence of the preparation factor, and in the event of influence, the corresponding variance estimate. The test of influence of the preparation factor is accom-

Table 2
Controlled factors for ANOVA

Factor	Type	No. of modalities
Batch	Random	n_{batch}
Preparation (batch)	Random	n_{prep} (for each batch)

Table 3
Theoretical analysis of variance table

Source of variation	Sum of squares: Q	Degrees of freedom: ν	Quantity estimated by the mean square: Q/ν
Batch	$Q_B = n_{inj} n_{prep} \sum_{j=1}^{n_{batch}} (\bar{X}_{j.} - \bar{x}_{..})^2$	$n_{batch} - 1$	$\sigma_r^2 + n_{inj}\sigma_{prep}^2 + n_{inj} n_{prep} \sigma_{batch}^2$
Preparation (batch)	$Q_C = n_{inj} \sum_{j=1}^{n_{batch}} \sum_{k=1}^{n_{prep}} (\bar{X}_{jk} - \bar{x}_{j.})^2$	$n_{batch}(n_{prep} - 1)$	$\sigma_r^2 + n_{inj}\sigma_{prep}^2$
Error	$Q_r = \sum_{j=1}^{n_{batch}} \sum_{k=1}^{n_{prep}} \sum_{\alpha=1}^{n_{inj}} (\bar{X}_{jk\alpha} - \bar{x}_{jk})^2$	$n_{batch} n_{prep}(n_{inj} - 1)$	σ_r^2
Total	$Q_T = \sum_{j=1}^{n_{batch}} \sum_{k=1}^{n_{prep}} \sum_{\alpha=1}^{n_{inj}} (\bar{X}_{jk\alpha} - \bar{x}_{..})^2$	$n_{batch} n_{prep} n_{inj} - 1$	σ_T^2

σ_r^2 = Residual variance.
σ_T^2 = Total variance.
σ_{batch}^2 = Inter-batch variance.
σ_{prep}^2 = Inter-preparation variance, and $\bar{x}_{jk} = \frac{1}{n}\sum_{\alpha=1}^{n} x_{jk\alpha}$ $\quad \bar{x}_{j.} = \frac{1}{nr}\sum_{k=1\alpha=1}^{r}\sum^{n} x_{jk\alpha}$ $\quad \bar{x}_{..} = \frac{1}{nrq}\sum_{j=1k=1\alpha=1}^{q}\sum^{r}\sum^{n} x_{jk\alpha}$

Table 4
Conclusions for the new laboratory

Source of variation	Significant influence (Y/N)	S.D. estimate	Degrees of freedom
Preparation	?	$\hat{\sigma}_{prep}$	$n_{batch}(n_{prep} - 1)$
Residual (repeatability)		$\hat{\sigma}_r$	$n_{batch} n_{prep}(n_{inj} - 1)$

plished by means of a Snedecor's test [19]. The value of the α level[1] must be determined in the transfer protocol. Values generally used are 1% or 5% [9,20–22]. The corresponding variance estimate is deduced from the mean square. To prevent discrepancies, it is strongly advised to test at this point the compatibility of residual precision between the reference entity and the new laboratory. In the event of compatibility, the use (both in tests and calculation) of the repeatability variance estimated from the inter-laboratory study allows a more powerful test of influence of the preparation factor, thanks to the higher number of degrees of freedom. In addition, it also allows a more reliable estimate of the preparation variance.

These conclusions are presented in Table 4.

3.3. Acceptance criteria for the new laboratory

In order to be validated, the new laboratory must fulfil both precision and accuracy requirements for each component of the product to be determined.

Repeatability of the new laboratory must not be significantly poorer than the reference repeatability determined through the collaborative study. The statistical null hypothesis is σ_r^2 (new laboratory) $\leq \sigma_r^2$ (collaborative study). The statistical test is a one-sided Snedecor's test whose discriminant function is $F = \left[\hat{\sigma}_r^2 \text{ (new laboratory)} / \hat{\sigma}_r^2 \text{ (collaborative study)}\right]$. If the numerical value of F is greater than the critical value of a Snedecor's variable with ν_1 and ν_2 degrees of freedom at significance level α, then the null hypothesis must be rejected. ν_1 and ν_2 are the degrees of freedom of the two variances' estimations; they are given in Tables 1 and 4, respectively. If this criterion is not met, the new laboratory must check its appliances in order to determine the origin of this insufficient repeatability.

The preparation criterion is to be tested only when the former criterion is met. It is in fact a precision criterion, but relative to the preparation factor. The inter-preparations dispersion of the new laboratory must not be worse than the reference inter-preparations dispersion, determined through the collaborative study. The statistical null hypothesis is σ_{prep}^2 (new laboratory) $\leq \sigma_{prep}^2$ (collaborative study). The statistical test is a Snedecor's test whose discriminant

[1] The α level involves a risk equal to α chances out of 100 that the null hypothesis will be rejected though it is true (type 1 error).

function is $F = \left[\hat{\sigma}^2_{\text{prep}}\ (\text{new laboratory}) / \hat{\sigma}^2_{\text{prep}}\ (\text{collaborative study})\right]$. If the numerical value of F is greater than the critical value of a Snedecor's variable with ν_1 and ν_2 degrees of freedom at significance level α, then the null hypothesis must be rejected. ν_1 and ν_2 are the degrees of freedom of the two variances' estimations; they are given in Tables 1 and 4, respectively. If this criterion is not met, the new laboratory must check its preparation technique and appliances (balance...) in order to determine the origin of this poor preparation technique. It is far better to test the significance of the preparation factor (and if necessary to estimate the corresponding variance) by using the repeatability variance estimate of the inter-laboratory study rather than those of the new laboratory.

The accuracy criterion will allow the new laboratory to assert that its results are free of bias. First, the new laboratory must analyze the certified batches of the collaborative study. The batch assay is then obtained by averaging all the results relating to the batch in the injection sequence. For each batch analyzed and for each component of interest in the product, the assays determined by the new laboratory must be within the confidence interval defined by Eq. (2).

$$\text{CI} = \left[T_c \pm t_{\alpha,\nu} \sqrt{\hat{\sigma}^2_{\text{lab}} + \frac{\hat{\sigma}^2_{\text{prep}}}{n_{\text{prep}}} + \frac{\hat{\sigma}^2_{\text{r}}}{n_{\text{prep}} n_{\text{inj}}}} \right] \qquad (2)$$

where T_c is the assay determined by the collaborative study, α significance level defining the risk of type 1 error; ν number of degrees of freedom that can be associated with the quantity under the square root; $t_{\alpha,\nu}$ critical value that a Student's variable with ν degrees of freedom has α chances out of 100 not to exceed; $\hat{\sigma}^2_{\text{lab}}$ estimate of the inter-laboratories variance in the collaborative study (see Table 1); $\hat{\sigma}^2_{\text{prep}}$ estimate of the inter-preparations variance in the collaborative study (see Table 1); $\hat{\sigma}^2_{\text{r}}$ estimate of the residual variance (reference repeatability) in the collaborative study (see Table 1); n_{prep} number of preparations (for each batch) used by the new laboratory (see Table 4) and n_{inj} number of injections (for each preparation) used by the new laboratory (see Table 4). By default, we shall choose the minimum of the number of degrees of freedom of the three variances' estimate. Generally speaking, it corresponds to the number of degrees of freedom of the inter-laboratories variance.

The accuracy is accepted if, for each component of interest in the product and for each certified batch, all the assays are within the corresponding intervals. If there is a batch whose assay is outside (or a component in a batch), an investigation must be carried out to determine the origin of the bias (matrix effect, insufficient resolution, etc.). It is true that with this criterion working too well during the collaborative trial can lead to rejection for a new laboratory. That is the reason why the laboratories involved in the collaborative trial have to be representative of the whole population of laboratories. The alternative approach using tolerated biases is, in our opinion, less rigorous because of its arbitrary content.

3.4. Transfer validation

Finally the transfer is validated when, for each component of interest in the product (drug substance and even related substances), and for as many certified batches as are necessary to obtain a representative set, all the above criteria are met. (i) The repeatability of the new laboratory is not significantly worse than the repeatability determined by the collaborative study. (ii) The preparation variance of the new laboratory is not significantly greater than the preparation variance determined by the collaborative study. (iii) The new laboratory presents no bias.

Fig. 1 illustrates schematically the whole transfer validation procedure.

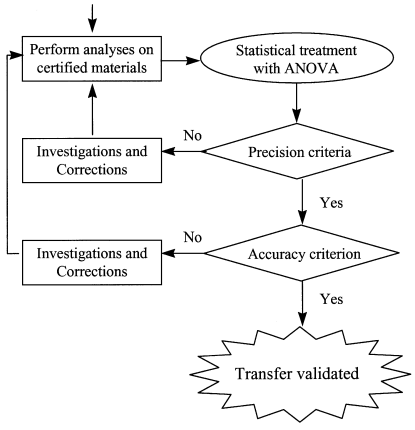

Fig. 1. Procedure for validation of a new laboratory.

4. Practical example

The example chosen was based on the results of the inter-laboratory study conducted on the spiramycin analysis [16,17]. Since it was merely an example, we have decided to describe the validation steps of the new laboratory only for the assay determination of a single component (spiramycin I) in the product. Nevertheless, if related substances' assays were also of interest, the data treatment method would be similar.

4.1. Preliminary collaborative study

Seven laboratories were involved. The number of batches was two, denoted P and Q. For each laboratory, the injection sequence was: three preparation×two injections. It is described below:

B, $R1_1$, $R2_1$, $R3_1$, $P1_1$, $P2_1$, $P3_1$, $P1_2$, $P2_2$, $P3_2$, $R1_2$, $R2_2$, $R3_2$, $Q1_1$, $Q2_1$, $Q3_1$, $Q1_2$, $Q2_2$, $Q3_2$, $R1_3$, $R2_3$, $R3_3$, B.

where B is the blank injection, R_{jk} *k*th injection of the *j*th preparation of the standard and P_{jk} (resp. Q_{jk}) *k*th injection of the *j*th preparation of the batch P (resp. Q).

Statistical exploitation of the results was undertaken using JMP software [23]. The conclusions and the results of the collaborative trials, for the main product, are presented in Table 5.

The assays in spiramycin I of the batches P and Q determined through the collaborative study were 86.23% for P and 85.94% for Q.

4.2. Experimental conditions

In this example, for validation of the new laboratory, certified batches P and Q of the collaborative study were used. Sample technique preparation was identical to that used during the collaborative study. The conformity tests were passed successfully.

The injection sequence used by the new laboratory was identical to the collaborative study sequence.

4.3. Data exploitation

The assay calculation methods for both the new laboratory and the collaborative study were identical and followed the US FDA recommendations.

$$T_{P_{jk}} = \frac{A(P_{jk})}{\left(\sum_{j=1}^{p}\sum_{k=1}^{n} A(R_{jk})\right)/9} T_R \quad (3)$$

where T_{Pjk} is the assay found at the *k*th injection of the *j*th preparation of batch P; $A(P_{jk})$ reduced area (i.e., area divided by the mass weighted) of the *k*th injection of the *j*th preparation of batch P; $A(R_{jk})$ reduced area of the *k*th injection of the *j*th preparation of the standard and T_R assay of the standard.

The controlled factors, both random, are the batch (two modalities) and the preparation (three modalities).

Table 6 shows the corresponding table for analysis of variance.

Quantities called ''sums of squares'' were computed using JMP software [23]. We chose to carry out our tests with a significance level of $\alpha = 0.05$.

The repeatability of the new laboratory was characterized by the residual standard deviation the estimate of which was 0.20. The repeatability variances of the new laboratory and of the collaborative study were compared by means of a Snedecor's test.

$$F = \frac{\hat{\sigma}_r^2 \text{ (new laboratory)}}{\hat{\sigma}_r^2 \text{ (collaborative study)}} = \frac{0.04}{0.15} = 0.26$$

This value must be compared with the value that a

Table 5
Conclusions of the spiramycin collaborative study

Source of variation	Significant influence (Y/N)	S.D. estimate	Degrees of freedom
Laboratory	Yes	$\hat{\sigma}_{lab} = 0.53$	6
Batch	No	$\hat{\sigma}_{batch} = 0$	1
Method/batch interaction	No	$\hat{\sigma}_{lab/batch} = 0$	6
Preparation	Yes	$\hat{\sigma}_{prep} = 0.73$	28
Residual (repeatability)		$\hat{\sigma}_r = 0.39$	42

Table 6
Analysis of variance for the example

Source of variation	Sum of squares: Q	Degrees of freedom: ν	Quantity estimated by the mean square: Q/ν
Batch	$Q_B = 2.21$	1	$\sigma_r^2 + 2\sigma_{prep}^2 + 6\sigma_{batch}^2$
Preparation (batch)	$Q_C = 3.49$	4	$\sigma_r^2 + 2\sigma_{prep}^2$
Error	$Q_r = 0.24$	6	σ_r^2
Total	$Q_T = 5.93$	11	σ_T^2

Snedecor's variable with 6 and 42 degrees of freedom has 5 chances out of 100 not to exceed. In tables we read $F_{0.05}(6, 42) = 2.32$. The null hypothesis could not therefore be rejected. The new laboratory's repeatability was not worse than that observed in the collaborative study. In this case, the use of a Snedecor's distribution table was not even necessary to draw such a conclusion since $F = [\hat{\sigma}_r^2$ (new laboratory)$/\hat{\sigma}_r^2$ (collaborative study)$]$ was less than 1.

The potential influence of the preparation factor was tested by comparing the inter-preparations mean square obtained for the new laboratory to $\hat{\sigma}_r^2$ (collaborative study). Here the value of the discriminant function of the Snedecor's test is $0.87/0.15 = 5.80$. This value must be compared with the value that a Snedecor's variable with 4 and 42 degrees of freedom has 5 chances out of 100 not to exceed. In the tables we read $F_{0.05}(4, 42) = 2.59$. The hypothesis of non-influence for the preparation factor must be rejected. The inter-preparations variance was calculated using equations given in Table 6. $\hat{\sigma}_{prep}^2 = (0.87 - 0.15)/2 = 0.36$. The S.D. estimate is the square root of this variance estimate: 0.60.

The preparation variances of the new laboratory and of the collaborative study were compared by means of a Snedecor's test.

$$F = \frac{\hat{\sigma}_{prep}^2 \text{ (new laboratory)}}{\hat{\sigma}_{prep}^2 \text{ (collaborative study)}} = \frac{0.36}{0.54} = 0.67$$

This value must be compared with the value that a Snedecor's variable with 4 and 28 degrees of freedom has 5 chances out of 100 not to exceed. In tables we read $F_{0.05}(4, 28) = 2.71$. The null hypothesis could not therefore be rejected. The preparation variance of the new laboratory is not worse than that of the collaborative study. Once again, it was easily concluded that the preparation factor was not significant, since the calculated ratio was less than 1.

The certified assays determined from the collaborative study were used for the accuracy criterion. The new laboratory's observed assays were 86.68% for batch P and 85.82% for batch Q.

For batch P the interval in which the assay had to be was: $\left[86.23 \pm 2.447\sqrt{0.28 + \frac{0.54}{3} + \frac{0.15}{3 \cdot 2}}\right] =$ [84.53; 87.93]

For batch Q the interval in which the assay had to be was: $\left[85.94 \pm 2.447\sqrt{0.28 + \frac{0.54}{3} + \frac{0.15}{3 \cdot 2}}\right] =$ [84.24; 87.64]

For batches P and Q the assays determined by the new laboratory were in the corresponding interval. Accuracy was validated.

In conclusion, the transfer was validated since, for the product concerned (spiramycin 1), and for both certified batches, all the criteria were fulfilled. (i) The repeatability of the new laboratory was not worse than the repeatability determined by the collaborative study. (ii) The preparation variance of the new laboratory was not worse than the preparation variance determined by the collaborative study. (iii) The new laboratory exhibited no bias.

5. How to proceed when certified materials are slightly degraded

When some certified materials are stored for a long period or under inadequate conditions, significant degradation may be suspected. In such a situation, using these certified materials for a transfer without precautions could be very hazardous. A problem arises when degradation reaches a level that leads to rejection of the accuracy criterion while there is no actual bias. To avoid such discrepancies, when there is a doubt concerning possible degradation of the certified materials, a special procedure

must be followed. Most results of the collaborative study are, however, still usable. It is worth noting that the approach is valid only in case of a slight degradation.

5.1. Reanalysis

The first step of the process consists in a reanalysis of the suspect certified material. This new analysis must be performed by one of the laboratories that took part in the collaborative study or at least by a laboratory validated using the protocol described above. This laboratory will be called reference laboratory. The experimental conditions and the injection sequence must be in accordance with those of the collaborative study. Data is processed by means of ANOVA. The model used is the same as that given in Eq. (1), and consequently the theoretical table is identical to Table 3.

The precision characteristics obtained in relation to the degraded certified material must be compared with those of the collaborative study. The procedure is the same as that described in Section 4.3. If the conclusions are that the dispersion characteristics (σ_r and σ_{prep}) are not statistically different from the dispersion characteristics obtained with the inter-laboratory study, then the dispersion values of the collaborative study can still be used with the suspected certified materials. If not, and on condition that the utmost care has been taken by the validated laboratory, it means that the degradation of the certified materials has profoundly modified the method dispersion characteristics. In this extreme situation there is no other solution than a new collaborative study.

It is also possible to check whether the degraded certified materials are actually degraded. This can be achieved using the accuracy criterion described at the end of Section 4.3. If the suspected certified materials are significantly degraded, the accuracy criterion will be rejected; otherwise the assays found in the collaborative study can still be used. We shall refer to the assays obtained by the laboratory that has reanalyzed the certified materials after degradation as T'_c.

The entire procedure that must be used with suspect certified materials is summarized in Fig. 2.

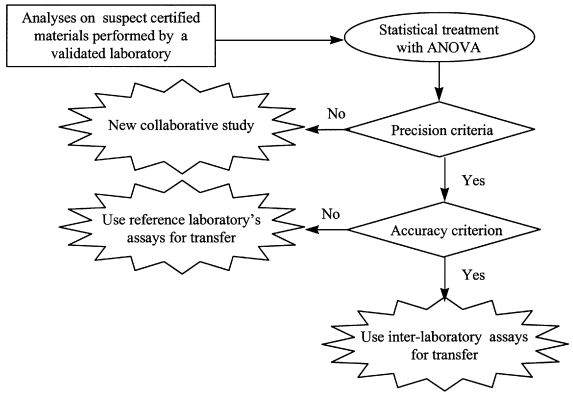

Fig. 2. Procedure with certified materials suspected of degradation.

5.2. Transfer to a new laboratory

When degradation is proved, the transfer protocol is identical to that described in Section 4. The only change concerns the accuracy criterion. Instead of the certified assays T_C determined by the collaborative study, henceforth the T'_c assays determined with a single laboratory must be used. Consequently the accuracy criterion must be formulated as follows: assays experimentally observed in the new laboratory must be in the confidence interval of Eq. (4).

$$CI = \left[T'_c \pm t_{\alpha,\nu} \sqrt{2\left(\hat{\sigma}^2_{lab} + \frac{\hat{\sigma}^2_{prep}}{n_{prep}} + \frac{\hat{\sigma}^2_r}{n_{prep} n_{inj}} \right)} \right] \quad (4)$$

where T'_c is the assay determined by the reference laboratory on the degraded certified materials; α significance level defining the risk of type 1 error; ν number of degrees of freedom that can be associated with the quantity under the square root; by default we shall choose the minimum of the number of degrees of freedom of the three variances. This generally corresponds to the number of degrees of freedom of the laboratory factor variance estimate. $t_{\alpha,\nu}$ is the value that a Student's variable with ν degrees of freedom has α chances out of 100 not to exceed; $\hat{\sigma}^2_{lab}$ estimate of the inter-laboratories variance of the collaborative study (see Table 1); $\hat{\sigma}^2_{prep}$ estimate of the inter-preparations variance of the collaborative study (see Table 1); $\hat{\sigma}^2_r$ estimate of the residual variance (reference repeatability) in the collaborative study (see Table 1); n_{prep} number of

preparations (for each batch) used by the new laboratory (see Table 4) and n_{inj} number of injections (for each preparation) used by the new laboratory (see Table 4).

Compared with Eq. (2), Eq. (4) is different by a factor 2 under the square root. This is because the assay of the candidate laboratory is no longer compared with a certified value (certified assay derived from the collaborative study), but with the realization of another random variable with the same dispersion characteristics.

If both precision and accuracy criteria are met the transfer is validated. This adaptation is a good way to ensure durability of the work accomplished during the collaborative study. It prevents a new full inter-laboratory study having to be undertaken when certified materials are subject to moderate degradation.

However, it must be borne in mind that such an approach is merely an adaptation, and consequently can in no sense aspire to the high level of rigor achieved by the standard protocol.

6. Conclusions

It has been clearly shown that it is possible to undertake an analytical transfer validation in quite a rigorous manner. Compared with the classical empirical method, a slightly higher level of investment is required. This investment is, in fact, the preliminary collaborative study. We should like to emphasize the term investment, since it is far from being a time loss. The collaborative study enables one to collect reliable data that can prevent endless discussions and waste of time or money in the event of difficulties. What is more, it is possible to adapt the transfer protocol even if the reference material exhibits relatively short time-stability. Another argument is that the collaborative study by itself does not represent an absolutely unrealistic venture for industrial companies: for each laboratory taking part in the study the time investment is in general two days of work.

To conclude, we should like to say that this methodology has already been put into application successfully by Rhône-Poulenc Rorer. However, this study can under no circumstances be published since the product used was a confidential one. The ultimate goal would be to apply a similar methodology for all kinds of transfers.

Acknowledgements

We would like to thank particularly Rhône-Poulenc Rorer's laboratories of Chemical and Pharmaceutical Development, which accepted to participate in these collaborative studies and to allow us some of their precious time. They are: Drug Safety Support Laboratories, Pharmaceutical Quality Analysis Department; Analytical Chemistry Unit Laboratories, Process Chemistry Department; Pharmaceutical Development Laboratory, Pharmaceutical Sciences Department.

References

[1] R.E. Pauls, R.W. McCoy, E.R. Ziegel, G.T. Fritz, D.M. Marmion, D.L. Krieger, J. Chromatogr. Sci. 26 (1988) 489–493.
[2] D.L. Massart, L. Perelgritz, Analusis 22 (1994) M13–M37.
[3] R.M. Venable, W. Horwitz, in: I.W. Wainer (Ed.), Liquid Chromatography in Pharmaceutical Development: An Introduction, Aster Publ. Corp., Springfield, OR, 1985, pp. 399–408.
[4] A. Marchetto, R. Mosello, G.A. Tartari, H. Muntau, M. Bianchi, H. Geiss, G. Serrini, G.S. Lanza, J. Chromatogr. A 706 (1995) 13–19.
[5] P. Parvy, J. Bardet, D. Rabier, M. Gasquet, P. Kamoun, Clin. Chem. 39 (1993) 1831–1836.
[6] J. Paesen, D.H. Calam, J.H.Mc.B Miller, G. Raiola, A. Rozanski, B. Silver, J. Hoogmartens, J. Liq. Chromatogr. 16 (1993) 1529–1544.
[7] K. Smets, E. Roets, J.McB. Miller, E. Porqueras, N. Berti, P. Vettori, F. Folliard, J. Hoogmartens, Chromatographia 37 (1993) 640–644.
[8] Ph. Quevauviller, D. Van Renterghem, B. Griepink, M. Valcarcel, M.D. Luque de Castro, J. Cosano, Anal. Chim. Acta 283 (1993) 600–606.
[9] H. Chung, M. Hsu, J. Chromatogr. 629 (1993) 277–281.
[10] L.M. Tsanaclis, J.F. Wilson, Clin. Chem. 39 (1993) 851–855.
[11] C. Hendrix, E. Roets, J. Crommen, J. De Beer, E. Porqueras, W. Van Den Bossche, J. Hoogmartens, J. Liq. Chromatogr. 16 (1993) 3321–3329.
[12] R.W. McCoy, R.L. Aiken, R.E. Pauls, E.R. Ziegel, T. Wolf, G.T. Fritz, D.M. Marmion, J. Chromatogr. Sci. 22 (1984) 425–431.

[13] R.E. Pauls, R.W. McCoy, E.R. Ziegel, T. Wolf, G.T. Fritz, D.M. Marmion, J. Chromatogr. Sci. 24 (1986) 273–277.
[14] R.E. Pauls, R.W. McCoy, J. High Resolut. Chromatogr., Chromatogr. Commun. 9 (1986) 600–601.
[15] C.L. Burgess, Clin. Res. Reg. Affairs 10 (1993) 137–157.
[16] A. Jardy, J. Vial, I. Ménier, Analusis 25 (1997) 106–111.
[17] J. Vial, I. Ménier, A. Jardy, P. Anger, A. Brun, L. Burbaud, J. Chromatogr. B 708 (1998) 131.
[18] Commissariat à l'Energie Atomique, Statistique Appliquée à l'Exploitation des Mesures, I et II, Masson, Paris, 1978.
[19] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Chichester, 2nd ed., 1988.
[20] Y. Vander Heyden, D.L. Massart, Y. Zhu, J. Hoogmartens, J. De Beer, J. Pharm. Biomed. Anal. 14 (1996) 1313–1326.
[21] Y. Vander Heyden, K. Luypaert, C. Hartmann, D.L. Massart, J. Hoogmartens, J. De Beer, Anal. Chim. Acta 312 (1995) 245–262.
[22] Y. Vander Heyden, C. Hartmann, D.L. Massart, L. Michel, P. Kiechle, F. Erni, Anal. Chim. Acta 316 (1995) 15–26.
[23] JMP Statistic and Graphics Guide and User's Guide (Version 3.1), SAS Institute Inc., Cary, NC, 1995.