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PII: S0308-8146(96)00245-2

Food Chemistry, Vol. 58, No. 1–2, pp. 145–147, 1997

0308-8146/97 $17.00+0.00
Analytical, Nutritional and Clinical Methods Section

LETTER TO THE EDITOR

How to present an analytical method

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(Received 1 July 1996; accepted 1 July 1996)

INTRODUCTION

Many chemists think that method validation is like a gold plating on a metallic tool: it shines but it is not essential to the function of the object itself. This sensation arises from reading papers being submitted for the publication in the *Analytical, Nutritional and Clinical Methods Section* of *Food Chemistry*: they often lack for significant information about method validation, or the validation data are added as an ornamental framework.

Two sections are necessary to provide a complete description of a method to be published: the first one should give essential information about materials and experimental procedures, in order to make the method repeatable by other laboratories or analysts. The second section should account for the method effectiveness. This validation procedure generally includes an evaluation of precision, linearity, and robustness and provides a measure of the method performance. The analytical method can be considered fully developed when it has been tested and found to show acceptable analytical performance.

Generally the papers being examined are informative enough about practical execution, but they are partially lacking in method validation.

Method validation is not a further refinement which must be carried on only when the method has to be applied as an official assay procedure, but it is an integral part of the method development routine: 'Method validation is the process of proving that an analytical method is acceptable for its intended purpose' (Green, 1996). If the purposes are well defined, i.e. the analyst knows the target compound levels, the acceptable level of confidence of the resulting data and the significant variation of the studied phenomena, then he must have complete knowledge of the accuracy and precision of his method, and be aware of the possible sources of errors as well. It is then obvious that method validation cannot be performed *a posteriori*, as it represents an integral part of the whole procedure and it depends on the specified purpose.

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Sometimes researchers develop a new analytical technique and then they look for an useful application in a certain applied field: then they publish their work in a scientific journal devoted to analytical chemistry. In these cases qualitative or semiquantitative results can be justified. However, when a paper is submitted for publication in an applied journal, such as *Food Chemistry*, the scientific problem should be well defined and the analytical method validated.

The aim of this note is to suggest the indispensable analytical and statistical parameters which are to be reported when a new analytical method is proposed.

ANALYTICAL METHOD VALIDATION: SOME PRACTICAL REMARKS

This short note does not claim to be a guide for method validation, as the reader can easily find a lot of books and papers on this topic. Furthermore, Food and Drug Administration (FDA) provides a framework to perform such validations (Food and Drug Administration, 1987), and the Association of Official Analytical Chemists (AOAC) published a well known manual (Youden & Steiner, 1975). In general, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, dynamic range, detection limit, quantitation limit and robustness.

In the *News and Feature Section* of *Analytical Chemistry*, a recently published paper (Green, 1996) defines all these topics very precisely following a practical approach: I do not want to repeat definitions or statistical principles, but I would like to make some observations on the use of these fundamental statistical parameters. The aim of this note is to answer the following question: what information does a reader need to find in a published method?

Specificity

The authors must describe the main characteristics of the analysed matrix, in order to prove the specificity

of the method for a certain analyte. This topic should represent the guide for the whole method development, with special regard to sample preparation and concentration. If the matrix is not well defined, the interference species cannot be recognised and taken into account.

Dynamic range

An adequate resolution between the analytes and the other components being obtained, it must be verified whether the sample solutions lie in the concentration range where the analyte response is linearly proportional to concentration. Validating over a wider range provides confidence that the routine standard levels are well removed from non linear response concentrations. Claims of linearity should be eyed with suspicion. *Instructions for Authors* of the *Journal of Chromatography* state that 'simple straight-line graphs (such as calibration lines) are not acceptable, because they can readily be described in the text by means of an equation or a sentence'. I do not agree with this sentence, because the calibration plots must always be inspected visually. They can evidence that a non linear calibration could be more representative, or that the linearity range is really less than that claimed. It is very important to show standard deviations in the linearity plots. However, even if standard errors are shown, weighted linear regression plots are not used, because common statistic softwares for PCs do not offer these facilities. The correlation coefficient is commonly used as a measure of the acceptability of linearity, but it is also easily misinterpreted. Some papers warn against using this coefficient (Miller, 1991; and references therein). An alternative way of evaluating data is to plot response factor [(peak area ratio–y intercept)/concentration] versus concentration. The variation of the resulting plot is a very straightforward mode to evaluate linearity.

After dynamic range assessment, calibration standard concentration must be defined in order to achieve the maximum accuracy in the sample concentration range. The range of an analytical method is, indeed, the concentration interval over which acceptable accuracy, linearity, and precision are obtained.

Precision and accuracy

The aim of an analytical method is to determine the 'true' value of a compound in a mixture or a matrix: so the precision and accuracy evaluation is essential and central in the method description. I have just read the following sentence in the well known *Standard Methods for the Examination of Water and Wastewater* at the end of the experimental section: 'Precision and bias data are not available' (APHA *et al.*, 1992). Therefore it does not appear obvious to remark that an analytical method cannot be used if it lacks for information about precision and accuracy. The final reproducibility and accuracy assessment of a method must be carried out by co-operative intercomparison tests, especially when the method is going to be used in official testing and controls. However, every researcher should perform some tests before using a new method, in order to verify whether it is effectively suitable to deal with his scientific problem.

Precision studies can range from the evaluation of the instrument or injection repeatability to the assessment of the inter-laboratory reproducibility. These precision studies are aimed to identify which factors contribute to the final results with significant variability, i.e. to evaluate the contributions to the total invariance due to the variances of the analytical steps, the sample, the equipment, the reagents and so on.

Once precision has been achieved, accuracy must be demonstrated: it is obvious that if a method is not precise, it can be neither accurate. Accuracy is usually determined using one of the following four ways: a) by analysing a sample of known concentration and comparing the measured value with the true one. This approach can be followed when reference standard materials or certificated samples are available from co-operative studies. The comparison cannot be done on the basis of a visual examination, but it should be carried out by means of statistical tests, from the simple Student's t-test to more complex ones. It is evident that the simplest statistical approach is valid only when the two values have comparable precision (evaluated for example using a F-test), because the t-test is often negative when the distribution is very large (i.e. the two distributions are not significantly different).

b) The second approach requires the comparison between the results obtained on a certain series of samples using the new method with those obtained from an existing method which is known to be accurate. The comparison is usually carried out by plotting the results of the new method versus those of the well established one. I think that this kind of plot can only put into evidence whether there are significant systematic errors in one of the two methods (i.e. when the slope of the regression equation is significantly different from unity). Furthermore, the use of the correlation coefficients to assess that the two methods are comparable, is not statistically significant, as I have pointed out previously. A more correct approach, which is simple as well, is to use a paired t-test on the series of data (Jeffery *et al.*, 1989).

When standard methods or reference materials are not available, an estimation of the accuracy can be carried out by c) recovery or d) multiple standard addition tests. The former technique is performed by spiking known amounts of analytes in a blank matrix, which can be either natural or synthetic. In the case of more complex matrices, as it generally happens in food analysis, multiple standard additions can represent an useful approach, which shows, however, a lot of limitations

to be considered: for example the repeatability of the spiking procedure; the accuracy of the measure of the very little amounts to be added; the different physico-chemical behaviour of the analytes originally bonded to the matrix and of the spiked ones.

Robustness

Once these validation studies are complete, it should be very useful to present data about the robustness of the method. The robustness of an analytical method can be defined as its ability to remain unaffected by small changes in laboratory conditions, environment, analysts, materials and reagents. This parameter is very important in evaluating the possibility to transfer the developed method to other laboratories. Full dynamic range evaluation and determination of the detection limits can add useful information about the extensibility of the analytical procedure to analogous determinations in matrices of different origins.

Quantitation limit

(LOQ) can be calculated as the analyte concentration giving S/N = 10, but it should be better to consider LOQ as the analyte concentration at which the precision of the method becomes unacceptable (e.g. RSD $< 20\%$); most of the papers do not show any figures of the analysis of the samples at these concentrations, which could be very useful to evaluate the actual sensitivity of the method.

CONCLUSION

This paper does not pretend to be a guide for method validation, as a lot of books and papers are available on this topic. I only desire to make some comments on the scientific correctness of the presentation of an analytical method in an applied journal. In fact, the publication of a paper in a refereed journal should not be considered the target of the work itself, but only a communication medium to the scientific community. Thus, the success of a new published method depends essentially on the completeness and clarity of the work itself. If necessary information is lacking, the paper must be rejected, and precious time and promising and useful information for the scientific world is lost with it.

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