THE USE OF RECOVERY FACTORS IN TRACE ANALYSIS

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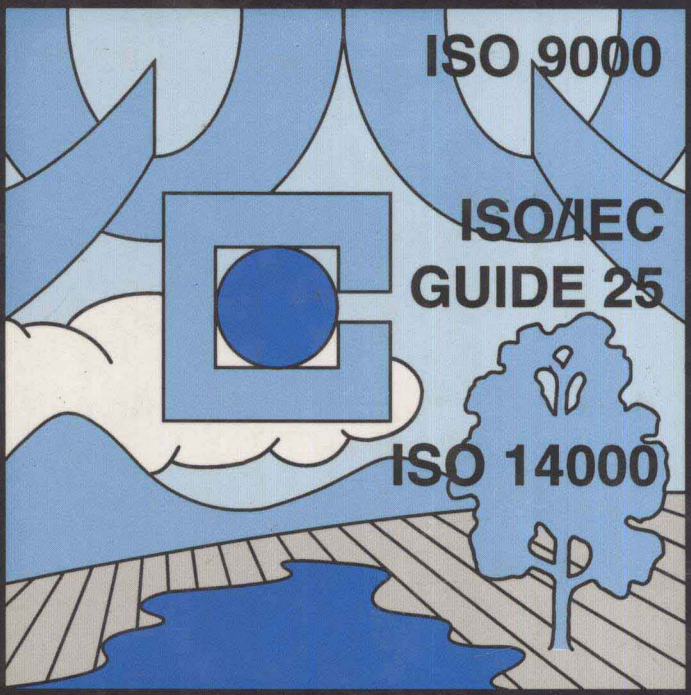

Edited by M. Parkany

The Use of Recovery Factors in Trace Analysis

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The Use of Recovery Factors in Trace Analysis

Foreword

by Dr. Lawrence D. Eicher, Secretary-General, ISO
(International Organization for Standardization)

This Seventh International Harmonization Symposium on the use of recovery factors is expected to examine all facets of the problem from the scientific, practical and legal points of view. Most of the lectures rightly focus on agricultural and food chemistry, since this is a problematic issue and the Codex Alimentarius Commission (CAC) expects a viable protocol (based on consensus) that they can adopt.

ISO is well aware that this problem has a detrimental effect on global trade in agricultural and food products. At this time there are no accepted standards or guidelines on recovery factors that might be followed. Furthermore, it is most unlikely that any single ruling could be applied in all cases. The practical examples mentioned by the lecturers will certainly help establish a list of problems together with analysis grouped according to the recommended or deprecated use of recovery factors.

In a certain number of lectures, cases are quoted where regulatory and/or court decisions depend on the application (or non-application) of recovery factors and their enormous economic consequences.

We standardizers know very well that soon there will be a demand to have clauses in the relevant international test method standards give unambiguous descriptions of recovery factors, and we are prepared for it.

There is no doubt that other analytical methods will have to be considered for the same problem. As an example, I mention human toxicology and specifically the field of Occupational Health and Safety (OHS) that I feel will soon be coming into the limelight.

It may be, however, that the most urgent demand will stem from the requirements of the first of the ISO 14000 series of environmental management standards on EMS (Environmental Management Systems) that will be published by the time of the Symposium. These standards, from the point of view of this Symposium, require valid and exact measurement methods, and clear indications on the use of recovery factors in order to establish traceable, documented facts able to stand up to any scientific scrutiny in the fields of

a) emissions to air;
b) releases to water;
c) waste management;
d) contamination of land;
e) impact on communities;
f) use of raw materials and natural resources;
g) other environmental issues.

Furthermore, following the EMS standards on the Continual Improvement Spiral, the Checking and Corrective Action Stage requires (among other actions) Monitoring and Measurement. No one will argue that valid, indisputable procedures have to be applied if the standard is to be implemented. Pushing this logic one step further, all will also agree that the correct use of recovery factors, as for example in the case of the analysis of contaminated land, has to be established for an implementation of the ISO 14000 series. How can auditors/assessors establish the implementation of these standards if correct measurements and calculations are not standardized? How can a company be certified to these standards if the cornerstone of *measurement* is not laid down ? I foresee strong pressures from the market to have such certification, and this in turn will create a demand for the Working Party on Harmonization to finalize the protocol on recovery factors as soon as possible.

Introduction

by Dr. Michael Parkany, Consultant, ISO
(International Organization for Standardization)

This is the seventh in the series of International Symposia for Harmonization in the field of Quality Assurance in Analytical Chemistry.

This Seventh Symposium (planned for 4-5 September 1996 in Orlando, Florida, USA) is devoted entirely to the subject: When is it justified to use recovery factors? It is evident that in this type of analysis the description of the analytical method must be clear on this and must give precise instructions. When preparing these Proceedings it was found that at present standards and protocols on analytical procedures very rarely fulfill this requirement. This is why it is so problematic to obtain a real picture of whether and when the use of recovery factors is forbidden, tolerated, encouraged or required. In fact, very often, the choice is left for the analyst in charge. Evidently judges in courts cannot accept such a situation, since they cannot base their judgements on conflicting interpretation of standards, neither are they in a position to improve the texts of standards and protocols for analytical procedures. They rightly require that these documents give clear instructions.

The lectures in these Proceedings review the present situation and offer practical solutions based on the experience of analysts of the field. Furthermore, a questionnaire was circulated to about eight hundred laboratories in order to establish present day practice. On the basis of answers to this questionnaire and of lectures at the Symposium, a Draft Protocol will be prepared by the IUPAC Interdivisional Working Party on Harmonization of Quality Assurance Schemes for Analytical Laboratories. This Protocol, when accepted, will be adopted by the Codex Alimentarius Commission (FAO -WHO). Finally a majority of countries will incorporate it into their legal systems.

This is why it is extremely important to be very careful when preparing a Protocol that will influence analytical results and important decisions in the field of food, agriculture, environment, toxicology, etc.

Standards writers should be involved and they should participate in the work, or at least be informed. Since the sponsors of the Symposium are ISO, IUPAC and AOAC International, the main international partners are involved.

I am very pleased that among the illustrious scientists involved we have the honour to have a lecture offered by Dr. William Horwitz, who has the longest career among us in this field and whom we regard as our Master/teacher. His experience is a rich treasure that he generously makes available to all of us.

I wish to acknowledge the help of the active members of the IUPAC Interdivisional Working Party, among them first of all Dr. Roger Wood and Dr. Michael Thompson who accepted the task of the preparation of the questionnaire, the evaluation of the answers and the drafting of the Protocol.

We all extend our thanks to the authors for providing their manuscripts,and to Mrs. J. C. Freshwater of the Royal Society of Chemistry for arranging this special publication.

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The Use and Misuse of Recovery Factors in Analytical Chemistry

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The use of a correction to a measured value (recovery factor) is appropriate only if the deviation is the result of a systematic error and the purpose is to compensate for a constant error in operations or methodology (bias). If both the measured value and the correction factor are subject to random error, the standard deviation of their combination is the square root of the sum of the squares of the two standard deviations, from the law of propagation of error. In such cases it is not appropriate to use a correction factor.

1. INTRODUCTION

At first glance, the use of recovery factors in analytical chemistry is attractive. Thinking in terms of the solubility of a precipitate, the obvious unleachable color left on a filter paper, or the undesirable presence of inorganic salts in organic dietary fiber, the immediate reaction is to "correct" for such unwanted effects. In fact the definition of "result of a measurement" in the "International Vocabulary of Basic and General terms in Metrology"[1] as the "Value attributed to a measurand, obtained by measurement," states in a note that, "When a result is given, it should be made clear whether it refers to: the indication, the *uncorrected* result, the *corrected* result and whether several values are averaged" [emphasis added]. It continues with definitions for "uncorrected result" and for "corrected result," as the "the result of a measurement before [after] correction *for systematic error"* [emphasis added]. Therefore, the use of a correction to a measured value is appropriate only if the deviation is the result of a systematic error.

2. EXAMPLE

Let us take a simple example. Consider that you make a determination of a pesticide residue, XCl, in a test sample and find 1.0 mg/kg. You also take an identical material not containing the analyte [the blank (control)], add 1.0 mg XCl/kg, perform the analysis, and obtain 0.9 mg/kg for this spiked material. Do you conclude that your recovery is 90%?

Table 1 *Example of Recovery Calculations from Two Determinations (No. 1 and 2) of a Residue of XCl and Two Determinations (No. I and II) of 1.0 mg/kg XCl Added to a Blank Matrix:*

Analytical Results, mg/kg, 1 = 1.0 I = 0.9
2 = 0.9 II = 0.8
ave. = 0.95 AVE. = 0.85

Combinations				Recovery,
Control	Minus	Test	= Loss	% loss
I(0.9)	-	1(1.0)	0.1	10
I(0.9)	-	2(0.9)	0.0	0
I(0.9)	-	ave.(0.95)	0.05	5
II(0.8)	-	1(1.0)	0.2	20
II(0.8)	-	2(0.9)	0.1	10
II(0.8)	-	ave.(0.8)	0.95	15
AVE.(0.85)	-	1(1.0)	0.15	15
AVE.(0.85)	-	2(0.9)	0.05	5
AVE.(0.85)	-	ave.(0.85)	0.95	10

The answer is: If you stop here, yes. But if you repeat either or both analyses, you immediately discover a calculation problem. If the second result on the test sample is 0.9 mg XCl/kg, what number do you use as the basis for your calculation? Further, if a second control determination gives 0.8, which of the possible combinations do you use? Table 1 gives the possibilities in excruciating detail, together with the additional possibility of using the averages.

Most researchers will agree that the last calculation, using the averages, gives the "best" estimate of a 10% loss or a 90% recovery *in the long run*. But with single determinations of the tests and controls, any of the other values are equally likely. Consequently the analyst could report recoveries for this determination of anything from 80 to 100%. If values 1 and I were reported by analyst A and values 2 and II were reported by analyst B, they would both probably blame the test samples as the cause of the discrepancies. Yet all of these values can arise from sampling normal distributions of analytical values with means of 0.95 and 0.85, respectively, and standard deviations of about 0.15. The answer then comes down to this: You can correct for bias in the long run, but you have no control over individual value corrections.

3. PROPAGATION OF ERROR CONSIDERATIONS

This example provides a simplified illustration of the law of propagation of error. If both values entering into a final result are subject to random error, the standard deviation of their combination is the square root of the sum of the squares of the two standard deviations. Further, if the input values are averages the standard deviation of the average must be divided by the square root of the number of values in the average. Therefore, the standard deviation of the combination will include this additional factor.

For the example given, and assuming a standard deviation of 10%,

$$s_{AB} = \sqrt{(s_A^2 + s_B^2)} = \sqrt{(10^2 + 10^2)} = 14(\%)$$

If each value is the average of 2 values, the standard deviation of the result is sAB divided by $\sqrt{2}$ = 10%. The effect of the law of propagation of error is offset here only by doubling the amount of work performed.

4. APPROPRIATE AND INAPPROPRIATE USE OF RECOVERY FACTORS

The use of recovery factors is appropriate only when the purpose is to compensate for a constant error in operations or methodology (bias). This situation is most frequently encountered in extractions, both solid and liquid phase. Although it would appear that the recovery is constant, i.e., the initial isolation-of-analyte step of an analytical method fails to extract a constant amount, the recovery test concentration is usually measured by a procedure that is just as variable as the determination of the analyte concentration as an unknown, although the quantity added is fixed. Application of a constant recovery factor under such circumstances may reduce bias (difference from the true value in the long run) at the expense of precision. When a corrected result is reported, the variability of the measurement of the recovery is propagated into the variability of the determination, resulting in a relative standard deviation (RSD) of 1.4 ($\sqrt{2}$) times the RSD of the uncorrected individual value. When an internal standard is used, the same considerations still apply. If the internal standard has been conducted through the entire procedure, as it should be, and if it is measured in the same way as the test analyte, it is subject to the same sources of random error.

In pesticide residue analysis, the average long-term recovery at the ppm (10-6) level is 85-90%. However, a recovery factor is typically not applied for several reasons:

(1) The recovery of controls conducted simultaneously is just as variable as the individual determinations, e.g., 70-110%. Reports and publications typically present both sets of data independently for use by reviewers as they wish.

(2) Regulatory specifications usually do not need "true values." The tolerances that have been established already have taken the recovery into consideration. They are promulgated after a review of data supplied by the petitioner (at least for pesticide and veterinary drug residues in the US) that have been obtained from plants and tissues by the same methods that will be used in enforcement. Therefore the specification has already compensated for the recovery. The net result of the use of a correction factor in these cases is to provide higher values for the residues than were intended.

This [preadjustment] is most easily seen in the use of empirical methods (CODEX Type I methods) in establishing food composition specifications. When a composition specification is established (e.g., moisture, fat) the method is usually specified so that the results obtained for enforcement will correspond to the values that have been established as the required limits *by that method.*

(3) Those methods that use internal standards have an apparent "built-in" correction factor. However, this correction may be deceptive when the internal standard is not carried through the entire procedure, especially when the internal standard is added after the native residue has been extracted.

One other term is important in the calculation of recovery factors--the value to be used in the denominator, when a multiplicative constant is applied. Recovery can be calculated on the basis of (1) only the added analyte, with the native analyte in the matrix ignored (designated as marginal recovery), and (2) the total analyte present, both native and added (designated as total recovery). When the quantity of analyte in the matrix (blank or control) is essentially 0, both calculations give the same value; when the native amount present [the incurred residue] is significant, the "method of additions" becomes appropriate to determine the quantity originally present in the matrix, so that the recovery effort is more than a mere additional measurement. In such a case, more quantities must be measured, resulting in additional variability from propagation of error and thereby diluting the bias-correcting effort. Thus the operation may become a self-defeating exercise.

There is a further complicating effect when biological systems are involved. In such a case the administrator is not interested in minor analytical niceties. The decision-maker is interested in recoveries of the parent compound in cases of feeding veterinary drugs to animals or applying pesticides to crops, whereas the analyst is interested in the recovery of the particular metabolite that is easiest to measure. Therefore the laboratory manager must be very sensitive to the nature of the recovery being reported. Do the recoveries reported properly reflect the compound of interest, or something far down the reaction chain?

There are several other technical problems with the concept of correction factors, such as the mathematics of a multiplicative versus an additive factor, and the statistical problem of a standard deviation that varies with the concentration added, which we will merely mention, but not discuss.

Therefore, what appears on the surface as a rather trivial exercise can have considerable ramifications. By no means does this discussion advise that recovery of added analyte determinations not be performed. On the contrary, recovery studies are an essential component of quality assurance systems. Rather the discussion suggests reporting recovery results separately to permit an administrator to correct or not, whichever is appropriate for the purpose.

5. REFERENCES

'International Vocabulary of Basic and General Terms in Metrology', Second Edition, International Organization for Standardization, Geneva, 1993.

Considerations in the Estimation of Recovery in Inorganic Analysis

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1. INTRODUCTION

Harmonized, reliable analytical measurements are vital in routine analysis, research, comparison of methods and information, standardization of laboratory performances and legal compliance with government regulations for national requirements and international trade. Inclusion of appropriate tests of analyte recovery into the analytical scheme, incorporating good methods and other aspects of a quality control and quality assurance program, is integral to the ability to properly assess, monitor and maintain good analytical data quality.

The determination and use of recovery factors is currently a controversial issue among analytical chemists. Various concepts are held and approaches directed, in organic and inorganic determinations, to the estimation and use of recovery factors. A great many parameters impinge on the philosophy and practice of the determination and use of recovery factors which must be considered and evaluated in an effort to arrive at a consensus of a harmonized best practice.

The aim of this paper is to deal with some of the important parameters and issues related to recovery of inorganic elemental analytes, and to put forth some ideas, philosophy and approaches to stimulate and provoke thought and discussion by attendees at the Symposium in their quest for a consensus for harmonization. It will focus on general considerations for the use of recovery materials, estimation of recovery based on added and endogenous analytes, calculation of recovery factors and associated uncertainties, the impact of method and laboratory bias/systematic error on recovery and the application of recovery factors. Although the ideas herein relate to recovery considerations in general, the emphasis is on inorganic analysis, determination of elemental content at macro and trace levels. Furthermore, while extractable or bioavailable elemental concentrations are also of interest to some analysts, only total elemental concentrations are addressed.

2. GENERAL CONSIDERATIONS FOR USE OF RECOVERY MATERIALS

2.1 Sources of Error in Analytical Methods

The general lack of agreement among analytical results from different analysts and laboratories arises from numerous factors influencing the validity and reliability of the final numerical results. These factors can be broadly categorized as presampling, sampling, sample manipulation and measurement. Other important considerations such as contamination control, data quality control and the analyst transcend the above boundaries.

Presampling factors,[1] as far as biological specimens are concerned, include genetic predisposition, long term physiological influences such as age, sex, geographical and environmental factors, diet, pregnancy and lactation, short term physiological influences including circadian rhythms, recent meals, posture and stress, seasonal changes including physiologic and climatic influences, postmortem changes including cell swelling, imbibition and autolysis, intrinsic errors involving medication, haemolysis, subclinical conditions and medical restrictions in sampling, and correct choice of target organ.

Sampling refers to the entire collection of steps and considerations in procuring the sample such as identification of the population, establishment of a sampling model and plan, meaningful, proper and representative sampling using appropriate collection techniques, storage, transportation, and reduction of the gross sample to laboratory sample.[2-4]

Sample manipulation encompasses material and solution storage, decomposition, extraction and separation of analyte, volumetric ware verification, calibration, technique of use, sample drying and/or moisture determination and dilution schemes. Examples of sources of errors are: analyte volatilization losses in dry ashing or wet decomposition, incomplete destruction of matrix, recovery and analysis of insoluble residue, alteration of oxidation state during decomposition and extraction, contamination from ashing aids, acids and reagents, contamination from and losses to decomposition vessels, and incomplete separation/extraction. Measurement refers to the steps quantifying the amount of analyte present and covers the actual technique of signal determination, calibration, matrix effect management, data handling and calculation. It deals with selection of proper analytical techniques, instrument optimization, optimization of determinative technique performance characteristics and utilization procedures, correction for physical, chemical and background interferences, selection of calibrants (starting material purity and composition, preparation techniques of stock and working calibrants, verification, dilution schemes), selection of calibration solutions (single analyte, composite, matrix matching) and calibration technique (calibration curve, bracketing for high precision). The penultimate step in the scheme of analysis, preceding data interpretation and final use, is data handling and calculation which entails data and information recording and calculation, calibration curve fitting and calculation techniques, interpretation and evaluation (controls, statistical treatment, data presentation).

It is no wonder that such an extensive collection of potential pitfalls seriously impacts on data quality and typically imparts to it substantial questions of validity. Tests with recovery materials can monitor and control, to a good extent, the performance of the collection of laboratory procedures subsequent to the point of introduction of the material. Errors arising from activities occurring prior to this point of introduction, such as sampling, preservation, storage and presampling considerations are generally impossible to

monitor by use of recovery tests.

2.2 Procedures for Use of Recovery Materials

The recovery material, which can be either a spiked sample or a native analyte - natural matrix Reference Material, can be incorporated into the analytical sequence in one of several different ways. The suggested, preferred mode is incorporation at random in the sequence of samples in the batch analyzed. In the case of spiked samples, those to be fortified and to be tested for recovery can be selected randomly using random numbers, spiked and again randomly included in the sequence. Alternatively, for logistic and convenience considerations, selected samples and their spikes can be physically adjacent to each other in the analytical sequence, or the spiked samples may be placed at either the beginning or end of the batch.

Selection of recovery level for monitoring recovery should consider the expected concentration of the analyte determined and the possible effect of concentration on recovery. As a first choice, the level should be at or near the enforcement limit or near the level actually present in the sample for most appropriate monitoring of method performance, particularly when dealing with highly homogeneous materials. Alternatively, the spike level can be a certain multiple of the limit of quantitation, limit of determination or limit of reporting. Recovery testing should be carried out at higher levels than the preceding limits if higher concentrations are anticipated in the material or there is a question of material inhomogeneity. If labour is a consideration (and it likely is under today's global fiscal constraints) single, rather than duplicate, spikes of different samples/materials are preferred instead of duplicate spikes of the same sample or material; additional effort should be devoted to covering a wider range of material undergoing analysis. Consideration of determination of recovery for each matrix type encountered in analysis or only on selected matrices, representative of the materials analyzed, depends on the criticality of the analyses.

2.3 Multielement Determinations

In many analyses nowadays, methods are geared toward multianalyte determinations for cost-effectiveness and in response to the requirements of clients. The determination of a recovery factor for one analyte, and its application to monitoring/correction of other analytes, assuming it to be constant and applicable to all analytes determined, is unsuitable and strongly discouraged. This is equally true whether dealing with different or related organic analytes or elements. Although there will be a semblance of similarity in chemical behaviour among related analytes throughout the various chemical reactions constituting the method, behavioral differences can be significant. Error types and magnitudes can be quite specific to each analyte. The various errors in sampling, sample manipulation and measurement impact differently on recovery of analytes from the sample bulk matrix. It is to be expected that collection techniques, sample storage and transportation, reduction of the gross sample to laboratory sample, sample manipulation, analyte volatilization during decomposition, incomplete extraction/separation, analyte retention by solid residue from incomplete destruction of matrix, alteration of oxidation state during decomposition and extraction, contamination, calibration, matrix effect management, selection of proper analytical technique, correction for physical, chemical and background interferences,

starting calibrant material purity and composition and specific calculation details can all impinge differently on each analyte of interest. Thus when multielement determinations are conducted it is vital to determine an individual recovery factor for each analyte.

2.4 Preliminary Requirements

In order to produce valid analytical data and to properly and cost- effectively make use of recovery, it is essential that compliance with several prerequisites be established with the principal ones being correct analytical method and quality control.

An appropriate analytical method must be applied to the task on hand, by appropriately qualified and trained personnel in a suitable physical and administrative environment. Suitable physical environment refers to the equipment, materials, reagents and laboratory conditions necessary for the proper execution of the method; suitable administrative environment includes understanding of and support for appropriate data quality by the analyst's supervisor and all other managers. The role of the analyst is of direct paramount importance; good analysis and good analyst go hand in hand. Analyst training, experience, familiarity with the problem on hand, skill, attitude, motivation and judgement are necessary for satisfactory solution of analytical problems.

Suitable quality control/quality assurance procedures should be routinely in use and the need for appropriately reliable analytical information must be recognized. The analytical system must be in a state of statistical control; ie operating optimally and consistently generating acceptable data.

When dealing with the determination of total concentrations of elements, that is, the sum of all the element concentrations in all material (sample) phases and molecular species, it must be ascertained that the method is in fact measuring all of the element. The sample decomposition procedure must bring into solution all of the material with no grains or insoluble fraction left behind (eg. Ihnat [5]). In addition, the element must be in the correct oxidation state required by the various chemical reactions constituting the procedure.

3. DETERMINATION OF RECOVERY BASED ON ADDED ANALYTE

3.1 Applicability of the Spiking Approach

Frequently determination of recovery is based on the addition of the analyte being sought to a sample of material being analyzed. In this approach, a known quantity of pure analyte is introduced at some stage in the analysis process, the sample/analyte combination is carried through the analysis and comparison of results with the baseline value determined for the sample gives an estimate of recovery. The nature of the added analyte, selected from available elements or compounds used for calibrant preparation, is not necessarily identical to or representative of the nature and form of the native analyte occurring in the natural material being analyzed. This consideration is true for both organic and inorganic constituents. Thus, in principle, recovery estimated in this manner is not strictly accurate and should be regarded as solely an estimate. With organic analytes, reliance on spiking with analyte(s) of interest is, at times, the sole alternative for recovery determination.

Dependence of measured recovery on the nature of the element is not expected to be