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REFERENCE METHODS IN ULTRA-TRACE ANALYSIS OF MILK PRODUCTS

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Abstract - Based on experiences with international standardization of analytical methods for milk products are described the problems and solutions of collaboratively developing and testing reference and other standard methods for trace analysis of milk products. Contaminations from laboratory equipment, chemicals and air must be eliminated and interferences corrected. Precautions traditionally applied but normally unmentioned are to be identified and described. To ensure wide application of the methods the use of dangerous chemicals are avoided as far as possible and demands for expensive equipment is modest. Collaborative studies are essential before methods are finally accepted, and the results important for the status of the methods. Reference materials are useful complements to reference methods.

INTRODUCTION

The most commonly used methods for analysis of milk and milk products are produced by the International Dairy Federation, IDF. At present, in 1983, there exist over 100 analytical IDF standards (1), and 10-15 new or revised standards are produced annually.

The main purpose of the IDF analytical standards is to enable different laboratories to produce comparable results when examining whether product specifications are met in international trade with milk products. But the standards also find widespread use by other types of examinations of milk and milk products.

Some of the IDF analytical standards are designated reference methods, other routine methods, recommended methods or simply methods. But because they are internationally tested and accepted they are all used as reference methods to some extent. The methods have obtained still wider use in later years when close cooperation has been established between IDF and the International Standardization Organization (ISO) and the Association of Official Analytical Chemists (AOAC). All analytical standards are now developed and tested jointly, and published by each of the organizations separately. In the following reference is only made to the IDF standards, but almost identical ISO and AOAC standards exist in most cases.

TRACE COMPOUNDS IN MILK AND MILK PRODUCTS

Most of the standardized chemical methods for milk products deal with determination of major milk components like fat, protein, lactose, mineral salts and organic acids, or additives like preservatives, neutralizers etc. Only a few standards concern trace and ultra-trace analysis, and they deal mainly with certain unwanted contaminants. These contaminants are elements like heavy metals, primarily copper (2) and iron (3) that are both causing oxidation if contaminating fatty milk products. The trace contaminant analyses also include standards for determination of residues of organochlorine pesticides (4) and aflatoxin (5). When such contaminants are present in milk it is because they have been transferred from feeding stuffs to the cow and secreted into the milk. Similar standard methods are in the process of being established for trace contaminants like lead, iodine, antibiotics and polychlorinated biphenyls, and for residues or

carry-overs of antioxidants and preservatives. Naturally there are many more trace compounds to be found in milk and milk products, but they are of less commercial and toxicological importance, and consequently there is less interest in developing international standard methods for their determination.

DEVELOPING REFERENCE METHODS FOR TRACE ANALYSIS: PROBLEMS AND SOLUTIONS

The international analytical standards are developed and tested by groups of experts appointed by the national organizations of IDF and ISO, and through the general referee of AOAC. Normally it takes a few years for such a group to establish a reference method, but in case of trace analysis it takes several years because it gives many more problems.

The characteristic problems and their possible solutions are reported in the following with typical examples originating from the experiences obtained in the IDF/ISO/AOAC Heavy Metals Group. The main problems are

- Contaminations from laboratory equipment, chemicals and air.
- Interferences from matrix.
- Losses by ashing.
- Lack of reference materials.
- Differences in laboratory tradition.
- Health risks from chemicals and equipment.
- Use of expensive instruments against use of time-consuming traditional laboratory procedures.

The first mentioned problems result in high blanks and inconsistent results indicating that the method used is not suitable for standardization. The latter mentioned problems makes it difficult to establish international methods that will satisfy different national conditions and requirements.

Contaminations from laboratory equipment, chemicals and air

By far the biggest problem in trace analysis is the risk of contamination of the sample. Most of these contaminations can be registered as high values of the blank samples, but blanks must be kept low if reliable results shall be obtained in trace analysis. This requires:

1) Use of ultra-clean, non-leaching glassware and other equipment.

The normal procedure for cleaning glassware for trace metal analysis is to soak the glassware in nitric acid, but better results can be obtained by steaming-out in closed systems (6), before storage in clean containers. Still, soaking in nitric acid has proved inadequate to prevent leaching of iron from glass beads when used as boiling aids by acid decomposition of samples for iron determination. Only the use of quartz beads can prevent high blanks. For this reason quartz or other inert materials are recommended for containers, boiling aids etc. by all heat treatments of samples. To avoid leaching it is also recommended to avoid use of coloured plastic tips for Finn-pipettes used in the analytical procedure. No effect in the direction of iron leaching has, however, been found by using brown in stead of clear glass containers for storage of reagents.

2) Use of very pure chemicals, if necessary purification of the chemicals shortly before use.

It is seldom mentioned in description of methods, but is common practice in most laboratories used to trace analysis of heavy metals in food, to purify the nitric acid yourself because even the best qualities commercially available tend to have or gradually acquire contaminating concentrations of heavy metals. Nitric acid is easily purified to suitable purity by sub-boiling distillation in quartz glass equipment (6).
Another observation is that although most trace methods demand that all water is double-distilled, few laboratories actually do this. In stead they distill de-ionized water once, but experience has shown this is often not enough. Two distillation units (preferably fully or partly made of quartz-glass) can, however, easily be coupled.

3) Precautions against airborne contamination.

Experience has shown that considerable amounts of iron may contaminate the

air for instance in fume hoods, where acid gasses make steel equipment rust. This contamination can be sufficiently reduced by covering rusty or otherwise corroded equipment with teflon tape, asbestos rope or other inert material. Contamination of laboratory air with mercury is a well-known risk, but it has also been observed that cadmium used in reduction columns for nitrate determination in one room can contaminate the air so much that it may seriously affect the results of cadmium determinations in another room 50 m away. On this background it is natural that it has become a valuable help in many dairy laboratories to use clean-benches with laminar flow of HEPA (high efficiency particulate air)-filtered air when dealing with trace analyses. Still only a couple of dairy research laboratories are using real clean rooms as recommended for ultra-trace analyses (6, 7).

4) Training of the laboratory personel.

It takes long time and requires special training of the laboratory technicians to introduce trace analysis in laboratories not used to this. Experience shows that it is easy to adopt the purification techniques, but more difficult to remember taking all precautions necessary to avoid contamination in every detail down to such trivialities as never to let a pipette in use lie on the table. However, once the tradition is well established it takes little to maintain.

5) Identification and elimination of contamination sources.

The simplest way to check that contamination is kept low is to analyse blanks regularly (8). In case of high blanks the contamination sources can be identified by systematic studies of the effect of every step in the procedure on the blank value. When identified measures can be taken to eliminate the contamination.

Interferences from matrix. Losses by ashing.

The matrix of milk products is a complex mixture of fat, protein, carbohydrate and mineral salts including large amounts of calcium and phosphate, and some chloride. This makes it relatively complicated to decompose milk products for analysis of inorganic traces. Acid digestion is slow, and dry ashing may give losses of volatile chlorides. The high contents of calcium in the matrix tend to give interferences by determining of heavy metals by atomic absorption spectrophotometry. The problem of volatilization can probably be solved by use of programmed heating, if necessary after adjustment of pH. But very few dairy laboratories own a programmable oven, and consequently dry ashing has not yet been prescribed in any of the internationally standardized reference methods for trace determination in milk products. A more sophisticated mechanized instrument for combustion of organic samples in pure oxygen, the Trace-O-Mat (9, 10), has been used experimentally for milk products. The instrument has given some problems but seems useful with a few modifications. Considerable concern has also been given to the general effect of the milk matrix on the analytical results, especially by use of standard addition techniques (11, 12). Apparently there is a strong influence of the matrix on the slope and zero points of the standard curve, and precautions are considered for prescribing adequate correcting and zeroing procedures in the standard text.

Lack of reference materials

Surprisingly no certified reference materials comparable to milk products are available from the American National Bureau of Standards (NBS) or the European Community Bureau of References (BCR). Reference materials based on milk powder are, however, on their way to be certified by BCR in collaboration with several dairy and other laboratories. Such a reference material would be of great help for checking reference and other standard methods, as well as instrumental and locally used methods.

Differences in laboratory traditions

Experience has shown that the text of a method succesfully used in one or more laboratories in one country, may give confusion or poor results if used in laboratories in another country. The reason is differences in laboratory language and traditions. But if a method is to become an international standard it is necessary that the text can be understood correctly in all other countries. So initial problems in understanding serve well for improvement of the text before it is finally published.

Health risks from chemicals and equipment

It is a problem to what extent certain chemicals and procedures shall be

regarded as dangerous and for this reason be rejected as unsuitable for use in an international standard. Particularly in two cases there has been doubt namely concerning the use of perchloric acid and the use of pressure vessels ("teflon bombs") for simple and rapid acid decomposition of organic material. Both systems could cause explosions if the right amounts of chemicals in proportion to the sample were not used or the pressure vessels not properly secured. In view of the risks for misunderstandings of the text (see above) by people not accustomed to such work it was decided to exclude this use of perchloric acid and "teflon bombs" from international standard methods for milk products.

Another problem is the increasing number of chemicals being regarded in some countries as so toxic that they should not be used in analytical work. Some of these chemicals cannot be substituted by others for their purpose. No solution has yet been found to this problem.

Use of expensive instruments against use of time-consuming traditional laboratory procedures

With more and more instruments being developed for more rapid, more sensitive and more automated determinations it becomes a question if the international reference methods shall demand use of the more modern techniques or refer to the traditional, time-consuming laboratory procedures. A final solution has not been found, but it is the general opinion that reference methods should be possible to carry out in as many countries and laboratories possible, which favours the traditional procedures. Rapid methods using sophisticated techniques could then be standardized as other types of method. However, these points of view may be revised. Ten years ago atomic absorbtion equipment was quite exclusive and was only gradually used to a larger extent in the majority of control laboratories. To-day HPLC is found only in few dairy laboratories, but this type of equipment seems to gain access more rapidly and to more laboratories than the atomic absorption equipment did. The future will show if this technique will become so widely spread that reference methods can be based on them.

TESTING REFERENCE METHODS BY COLLABORATIVE STUDIES

During the preliminary work on selecting the most suitable method among several candidate methods, and trying out the potential of this method usually a number of laboratories in different countries will be carrying out the first collaborative testing of the method. When the work reaches the stage where the method and the text describing the method seem ready, a proper collaborative study is organized among the members of the group of experts, if necessary including more laboratories.

The collaborative studies are organized after the principles described by ISO (13) or AOAC (14) with individual modifications. If the result of the collaborative study is satisfactory the method is proposed for publication as a provisional standard. The results of the collaborative study will be published separately and reference made to this publication in the bibliography of the standard.

It remains to be established how good the results of a collaborative study of a reference method on trace analysis should be. Horwitz, Kamps & Boyer in their paper on quality assurance in the analysis of foods for trace constituents (15) illustrate the rapid increase of the interlaboratory coefficient of variation as a function of the concentration of the trace constituent (Fig. 1). It is concluded that reasonable criteria for between-laboratory reproducibility (expressed as the coefficient of variation) of practical analytical methods in common use for monitoring residues and contaminants of public health significance are 16 % at the mg/kg (ppm) level and 32 % at the µg/kg (ppb) level. For metals like copper, zink, lead and cadmium at low levels the coefficient of variation tends to increase even more and this should all be taken in consideration when evaluating the result of a collaborative study.

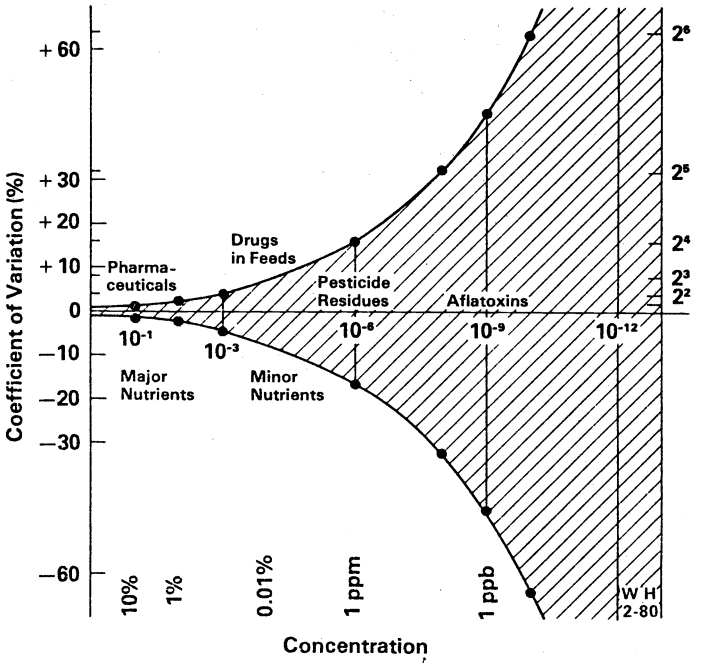

Fig. 1 Interlaboratory coefficient of variation as a function of concentration. (Horwitz, Kamps & Boyer (15).

REFERENCE METHODS AND REFERENCE MATERIALS FOR TRACE ANALYSIS

Because it proved difficult to meet the demands for reference methods based on all the very different instruments that are used for heavy metals analysis, the IDF/ISO/AOAC Heavy Metals Group already in 1979-80 (16) engaged itself in work on developing reference materials, for use instead of or for supplementing reference methods. Contact was established with BCR in 1981 and the above mentioned work started. This may lead to certification of milk-based reference materials certified for their contents of trace constituents of common interest.

Horwitz, Kamps & Boyer (15) also recommend the use of reference materials in connection with trace analysis of food, stressing that analytical work at trace levels must be constantly monitored through analysis of reference materials.

CONCLUSION

The result of the joint work of the three organizations (International Dairy Federation, International Standardization Organization, Association of Official Analytical Chemists) show that it is possible but cumbersome to establish international reference methods in ultra-trace analysis of milk products.

A number of sources of contamination were identified, and national diferences in laboratory tradition were eliminated by clarifying the text. Final collaborative studies demonstrated the validity of the methods.

Milk-based reference materials are expected to become important supplements to the reference methods.

REFERENCES

1. International Dairy Federation, Catalogue (1983).

2. International Dairy Federation, International IDF Standard 76 A (1980).

3. International Dairy Federation, Provisional International IDF Standard 103 (1981).

4. International Dairy Federation, Provisional International IDF Standard 75 A (1980).

5. International Dairy Federation, Provisional International IDF Standard III (1982).

6. P. Tschöbel, L. Kotz, W. Schulz, M. Veber & G. Tölg, Fresenius Z. Anal. Chem., 302, 1-14 (1980).

7. J.R. Moody, Analytical Chemistry, 54, 1358A - 1376A (1982).

8. R.K. Skogerboe, J. Assoc. Off. Anal. Chem., 65, 957-964 (1982).

9. G. Knapp, S.E. Raptis, G. Kaiser, G. Tölg, P. Schramel & B. Schreiber, Fresenius Z. Anal. Chem., 308, 97-103 (1981).

10. S.E. Raptis, G. Kaiser & G. Tölg, Analytica Chimica Acta, 138, 93-101 (1982).

11. E. Jackwerth, CLB Chemie für Labor und Betrieb, 33, 4-10 (1982).

12. K. Camman, Fresenius Z. Anal. Chem., 312, 515-516 (1982).

13. International Standardization Organization ISO, International Standard 5727 (1981).

14. W.J. Yoden & E.H. Steiner, Statistical Manual of the AOAC (1975).

15. W. Horwitz, L.R. Kamps & K.W. Boyer, J. Assoc. Off. Anal. Chem., 63, 1344-1354 (1980).

16. H. Werner, International Dairy Federation, E-Doc 138 (1981).