

Qualitative or quantitative methods for residue analysis?

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Qualitative methods are used for illegal drugs (e.g. hormones) which have a so-called zero tolerance (i.e. the presence of even a very small amount is not tolerated). In practice a limit of determination is used to prove the presence or absence of an analyte. Quantitative analysis is necessary especially for residues with a maximum residue limit (MRL). The method used in this case must have a limit of quantification (much) lower than the MRL. In any case, quality criteria must be fulfilled before quantification. In this investigation the pro's and con's of different strategies towards the use of qualitative and quantitative methods are discussed from the point of view and the daily experience of routine control in two field laboratories and one national reference laboratory.

1. Introduction

The results of residue analysis in veterinary food inspection are becoming increasingly important. This is mainly due to the stricter legislation for some residues. Therefore the highest performance is required from the control methods.

Sometimes, the interpretation of analytical results has been viewed differently by inspection services and laboratories. Inspection services are interested mainly in a 'yes/no' answer: has this animal been illegally treated with anabolics?; is the concentration higher than a certain value (maximum residue limit (MRL), limit of determination (DtL))? etc. In fact, all questions may be reduced to one: has the law been violated? When the answer is yes, actions are taken: the animal is rejected, animals are seized on the farm, etc.

Laboratories evaluate crude analytical data on the basis of predefined criteria for all relevant parameters (e.g. S/N ratios, deviations from observed and target values for reference materials, etc.). For residue analysis on anabolic compounds such minimum quality criteria are part of EU legislation [1]. On the basis of these criteria they arrive at yes/no answers. The discussion on the kind of quality criteria for the conversion of analytical results into yes/no answers is not yet finished. Therefore, in most countries a system of first and second analyses is used. When the final answer of the first analysis is yes, legal action is suspended until the results of the second analysis, if any, are known.

If there is a contradiction between the first and the second analyses, the final result will be negative ('no' answer) in the case of screening (first) and confirmatory (second) analyses since the latter is, in terms of reliability, superior to the screening method. In the case of equivalent (confirmatory) methods, the agreement of the criteria for qualification (identification) and quantification becomes extremely important. This will be even more the case if there is a contradiction between EU member states, in which case arbitration becomes necessary.

2. Qualitative analysis

Qualitative methods may be used for illegal analytes having a so-called zero tolerance. If the analyte is detected, the concentration can be determined or estimated and an action limit (AL) is

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used to convert the results into 'yes' (positive) and 'no' (negative) answers. The AL is an agreement between inspection services and laboratories. Based on this limit (legal) action is taken.

Even though different laboratories, for very practical reasons, as a rule use different methods, the presence and the identity of an analyte must meet the right criteria: retention times or R_f values, specific colors, (mass) spectrometric data etc. The quality criteria accepted by the EU have been described earlier [1-4] (e.g. for mass spectrometry four ions with correct relative intensities are needed). In order to fulfil all these criteria, to see a spot or to separate a signal from the noise, a minimum amount of analyte will be necessary corresponding to a minimal mass content in the original sample (this is not necessarily $2 \mu\text{g/kg}$, the AL that is proposed for anabolics in the EU at the moment). Therefore, qualitative methods have an intrinsic (semi-)quantitative character.

When working under a quality system, e.g. based on EN 45001, validation of the standard operating procedures (SOPs) at the level of the AL is mandatory. The relation between the AL and the various analytical (and other) definitions of limits is not unequivocal. In analytical chemistry the practical limit of detection (sometimes called decision limit) and the theoretical limit of detection (mostly called limit of detection) are defined as respectively background+1.64s and +3s [5]. Moreover, in EU documents [1] the decision limit is defined as background+6s. The DtL is defined as the smallest quantity which may be detected according to all the quality criteria especially for specificity [1,6]. Inspection services sometimes define a decision limit as the limit on which a decision is taken. These different definitions and points of view make the dialogue between labs and inspection services not always easy. A scheme of the situation for the owner, the laboratory and the inspection services is given in Fig. 1.

The DtL is analyte and matrix dependent mostly in a low concentration range (e.g. 0.5 or 2 ppb). If below the AL, it may vary from lab to lab and from analyte to analyte without influencing the yes/no answer to the inspection services. In the follow up of contradictions between field laboratories the national reference laboratory (NRL) has to take this phenomenon into account. There is a possibility that the first lab, with very sensitive equipment, has given a result below the AL. However, this result may not be reproduced by a second lab of which the method is validated down to the AL but

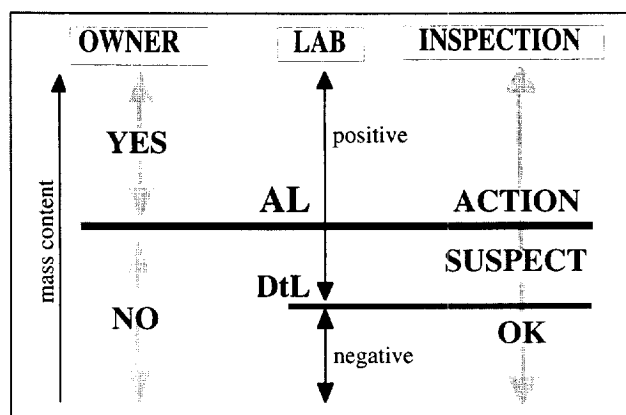


Fig. 1. The impact of the AL and the DtL on the owner of the animal and the Inspection services.

because of less sensitive equipment not to the DtL of the first laboratory.

3. Quantitative analysis

Quantitative analysis is necessary for residues with a MRL. The method used must have a limit of quantification lower than the MRL. Recently consensus was reached on the fact that analytical methods to be used for checking an MRL should have a limit of quantification of (at least) 0.5 MRL. For values (much) lower than the MRL, positive qualitative errors do not play a role in the 'no' answer. For values obtained in the relatively high concentration area of the MRL (e.g. 500 ppb) quantitative accuracy and reproducibility are of major importance. If the result lies above the MRL qualitative criteria must be taken into account for quantification.

The difference between a MRL and an AL is 3-fold. First, the MRL is mostly in a higher concentration range than an AL (e.g. MRL for some veterinary drugs = 100 ppb while AL for anabolics = 2 ppb). Second, MRLs are only applicable to residues of registered veterinary drugs in those edible matrices which are not prone to temporary fluctuations in concentration (there are MRLs for liver, meat, milk but not for urine; there are no MRLs for illegal substances). Third, a MRL is a legal value based on toxicological data while the AL is a value which is mostly an agreement based upon the analytical possibilities of the moment.

Three possible cases must be discussed.

(1) For values (much) lower than the MRL, positive quantitative errors do not play a role in

taking action. However, negative quantitative errors may be important. If a screening method is used (e.g. the four plate method for antibiotics, an immunoassay for β -agonists) some analytes may escape the law because the method has no or a much lower response to that analyte. However, this problem must be studied in the strategy of screening and confirmatory methods.

(2) If the result obtained lies above the MRL then qualification and quantification are very important. For surveillance of registered veterinary drugs immunological methods are often used. However, these are prone to cross-reaction which may influence the result. It is therefore important to confirm and quantify the results of immunoassay methods with an independent (chemical) method.

(3) For values in the neighborhood of the MRL, quantitative accuracy and precision become of extreme importance. Next to point 2, described above, a laboratory must provide a so-called safety zone depending upon the precision of the methods used there and in all possible second labs. In fact, a kind of secondary action limit (AL2) is created: $AL2 = MRL + 1.64 (s/\sqrt{n})$ (n = number of experiments). Moreover, it may be questioned how the quantitative result is to be converted into yes/no answers by rounding: will 1.56 ppb become 1.6 ppb (no) or 2 ppb (yes)? Most scientists give the significant figures (all certain figures + the first uncertain figure). The number of significant figures must reflect the precision of the analysis.

In any case, it should be clear that qualitative criteria must be fulfilled during quantification. Moreover, the manner of quantification is very important. When mass spectrometry is used the quantitative result may be calculated by using the full signal of the sample versus that of the internal standard (calibrated against a series of standards) (Fig. 2). Alternatively the sum of the three (or four) diagnostic ions, the most important ion or an algorithm including the correct ratios of the ions may be used. All possibilities will give different results and can be the cause of contradictions. In Fig. 2 peak 2 of the sample is distorted by an interference: taking the blind sum of all peaks will result in false quantification. A correction for the correct peak ratios should be made.

In real-life situations different laboratories will always use different methods (for calculation) and obtain different results: therefore a 'safety zone' is necessary.

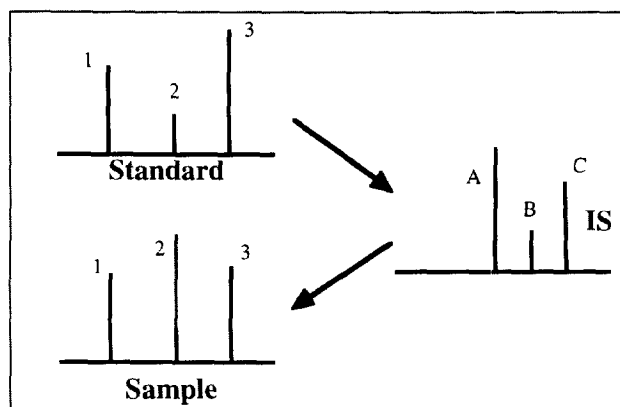


Fig. 2. Possible ways of quantification with mass spectrometry: blind sum of peaks 1, 2, and 3 in standard and sample to those of the internal standards A, B, and C or the use of correct peak ratios (in the sample peak 2 is distorted).

4. Interpretation of results

The problems described above are illustrated by the following example: in a farm 20 samples of urine are taken for analysis of anabolics (i.e. an illegal substance, so no MRL but an AL). In 16 of the 20 samples lab A detects the presence of an anabolic XG. However, in only one sample the concentration of XG exceeds the AL of 2 ppb for anabolics although for all 16 samples a fitting full spectrum or four diagnostic ions at the correct ratios were obtained. Which strategy should be followed?

(1) A 'yes' (positive) answer on one sample because the concentration is above the AL (2 ppb)?

(2) A 'yes' (positive) answer on all 16 samples (because quality criteria are fulfilled)?

In strategy 1, which is the less probable, the analysis may be contested because 19 animals were declared negative and only one positive. The farmer may declare that all animals belong to one box and that he does not understand why only that particular animal should be positive. In court (some years later) lawyers may focus on the 19 negative results and try to find an alternative cause for the one positive. First of all it should be emphasized (to non-analysts) that the meaning of the word 'negative' or a 'no' answer does not necessarily mean that the analyte was absent. On our analysis reports the meaning of the abbreviation 'neg' is explained on the basis of the EU regulations: neg = analyte absent or concentration lower than the 'action limit' [1].

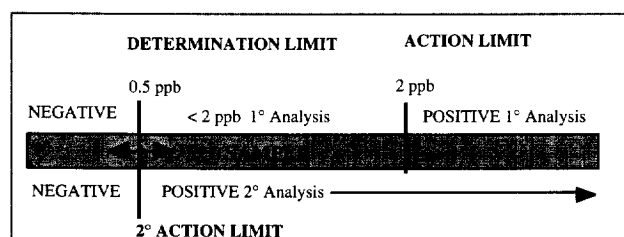


Fig. 3. Presentation of a double AL system in residue analysis. The (first) AL is fixed (e.g. 2 ppb). The second AL may vary with the DtL of the second laboratory.

In strategy 2 the analysis could be contested by the owner by selecting some animals (e.g. five) with the lowest concentration of XG and demanding a second analysis in an independent lab (the owner mostly knows very well the pharmacological history of his animals).

The following possibilities may occur.

(1) In the second analysis the second laboratory detects XG in all samples in a concentration range above the AL; this is a quantitative but not a qualitative contradiction with no consequences.

(2) The second lab does not detect XG. This may be due to a qualitative error in the first analysis (false positive) or because the concentration of XG is below the DtL of the second lab. This is a quantitative and/or qualitative contradiction.

(3) XG is detected by the second lab but in a concentration below the action limit (e.g. 0.4 ppb). According to the rules, the lab may decide that the sample is negative because it is below the AL. This is also a qualitative contradiction for the inspection services.

Contradictions of the second kind must be handled by the NRL. In the EU it is the task of the NRLs to check the results of the field laboratories.

Contradictions of the third kind could be avoided by introducing a double action limit system. This is represented in Fig. 3.

A laboratory carrying out a first analysis should make a decision based on the AL (e.g. 2 ppb). In samples containing analytes at a higher concentration than the AL the analytes found (but not the concentration) are mentioned. Samples below the AL are declared as 'no' answers in the light of administrative agreements (i.e. the risk of a negative result at second analysis becomes too high). The concentration range between the AL and the

DtL may be used as a warning or suspect zone: inspection services may be informed (off the record?) that illegal substances have been detected but at a concentration below the AL (Fig. 1). The inspection services may use that information for future sampling.

In a second analysis the confirmatory method with the lowest DtL of the second lab is used as an AL. That second AL is identical to a scientific parameter (DtL), which is laboratory dependent and not a consensus like the first AL.

A system with double ALs as described in Fig. 3 is only applicable to illegal substances with zero tolerance and thus not to MRLs.

5. Conclusions

Because of the AL, qualitative results cannot be produced without some form of quantification. Also in typically qualitative methods such as HPTLC some kind of semi-quantitative observation must be made.

The production of quantitative results without qualification is absurd. The analyst should always be sure of the identity of the molecule(s) for which the figure is produced. So, in our opinion the answer to the question "Qualitative or quantitative methods for residue analysis?" cannot be given that easily but will depend upon the legislation, the matrix and the analyte.

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book reviews

Accreditation and quality assurance

Accreditation and Quality Assurance in Analytical Chemistry, Edited by Helmut Gänzler (Translated from the original by Gaida Lapitajs), Springer, 1996, xvi + 266 pages, DM 148.00, ISBN 3-540-60103-1,

Navigating one's way round accreditation schemes in Analytical Chemistry is not for the faint-hearted. It is not so much the standards themselves. The EN 45000 series and ISO Guide 25 provide a firm point of reference, and the more broad minded can glance at ISO 9000 and the various Good Practice standards (GLP, GMP, GCP etc). Few can argue against the fundamental principles they contain, and those who do are probably pining after the days of alchemy. Modern science does not tolerate the secret ingredients and magic formulae which typify the romantic era of secret laboratories. The buzz-words are now 'standardisation' and 'reproducibility' — and quite right too!

No, the problem is not the standards. The problem is how they are implemented. From these core standards have sprouted positive forests of accreditation schemes addressing different situations in different countries.

Chapter 2 alone makes this book valuable as a reference guide. This is the most comprehensive map I have encountered to assist a tour round the

major accreditation schemes in Europe. From Austria to the UK the reader is given details of the what? how? and by whom? of the national accreditation schemes. Compare, for example, the wonderful complexity of the Italian approach with the direct simplicity of the Irish. Contrast the developing Russian approach with the systematic German structure. Why do we need so many bodies? The answer is not obvious, but the consequences are clear. To quote Hartwig Berghaus, "This leads to a lively Euro-tourism in the field of testing, certification and accreditation."

National mechanisms for accreditation may vary but there is a marked similarity in the content of the standards. "*Accreditation and Quality Assurance in Analytical Chemistry*" covers them in detail. Perhaps some of the guidance is occasionally over the top, but it is, most certainly, comprehensive. Chapters on Quality Assurance, Statistics and Validation deserve particular mention and the use of standards and reference materials receives no less rigorous treatment.

Not that the book is flawless. A general weakness is that it is a translation of a German original. On one hand it (necessarily) reflects the German approach to 'quality'. This may appear somewhat bureaucratic at times and the reviewer would prefer a

balance between the system and the scientist. Science is, ultimately, a human, creative process and cannot be reduced to a set of infallible rules. On the other, the generally excellent translation does fail at times. Usually this is trivial (there is a reference to 'meat requirements' which is more likely to be a comment on conformance than BSE!). Sometimes it is more confusing, and the English language has been extended by a few neologisms.

Specifically, some of the information is too specific to Germany. This is most obvious in the chapter on GLP. Requirements regarding, for example, GLP certificates and storage of SOPs are not universally applicable. It is unfortunate, in a book which takes such an international view of accreditation, that a such a narrow approach is adopted to GLP.

Perhaps it is churlish to raise such comments. They are minor in the context of a book which attempts, generally successfully, to place some logical structure in an area of administrative entropy. It has a valuable place on the shelves of anyone interested in Accreditation and Quality Assurance in Analytical Chemistry, providing that they use it selectively and do not try to adopt it literally in its entirety.

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