Trends in the identification of organic residues and contaminants: EC regulations under revision

François André
LDH-LNR, Ecole Nationale Vétérinaire, P.O. Box 50707, F-44087 Nantes Cedex 03, France

Katia K.G. De Wasch, Hubert F. De Brabander*, Sandra R. Impens
Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Food Inspection, Laboratory of Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium

Linda A.M. Stolker, Leen van Ginkel, Rainer W. Stephany
RIVM: European Union Community Reference Laboratory, A. Van Leeuwenhoeklaan 9, P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands

Robert Schilt
TNO Nutrition and Food Research, Department of Residue Analysis, Product Group Hormones and Veterinary Drugs, P.O. Box 360, 3700 AJ Zeist, The Netherlands

Dirk Courtheyn
State Laboratory Ghent, Braemkasteelstraat 49, B-9050 Gentbrugge, Belgium

Yves Bonnaire
Laboratoire du LAB/FNCF, Avenue de la division Leclerc 169, F-92290 Chatenay Malabry, France

Peter Fürst
Chemical and Veterinary Control Laboratory, D-48151 Münster, Germany

Petra Gowik
BgVV: European Union Community Reference Laboratory, Diedersdorfer Weg 1, D-12277 Berlin, Germany

Glenn Kennedy
Department of Agriculture and Rural Development, Veterinary Sciences Division, Stoney Road, Stormont, Belfast BT4 3SD, UK

Thomas Kuhn
Federal Veterinary Institute, Robert Koch Gasse 17, A-2340 Mödling, Austria

Jean-Pierre Moretain
AFSSA-Laboratoire d'Etudes et de Recherche sur les Médicaments, Vétérinaires et les Désinfectants, P.O. Box 90203, Javené, 35302 Fougères, France

Maurice Sauer
Department of Risk Research, Veterinary Laboratories Agency, New Haw, Addlestone KT15 3NB, UK

*Corresponding author. Tel.: +32 (9) 2647460; Fax: +32 (9) 264 7492.
E-mail: hubert.debrabander@rug.ac.be

0165-9936/01/$ – see front matter
PII: S0165-9936(01)00085-1

The use of identification points (IPs) is a new approach to set up quality criteria for the identification of organic residues and contaminants: a laboratory is allowed to use any molecular spectrometric technique or combination of techniques in order to earn a minimum number of points. The system of IPs balances the identification power of the different analytical techniques and has the advantage that new techniques can be introduced very easily.

Keywords: Residue; Veterinary drug; Analysis; Quality criterion; Identification point; Legislation; Contaminant

1. Introduction

In the European Union the European Commission regulates the inspection of animals and fresh meat for the presence of residues of veterinary drugs and specific contaminants (Council Directive 86/469/EEC, subsequently replaced by 96/23/EC) [1,2]. The quality criteria for the analysis of such residues are described in a series of Commission Decisions [3,4]. These legislative decisions are revised periodically to take into account current scientific knowledge and the latest technical improvements. The two Decisions effective since April 1993 are 93/256/EEC and 93/257/EEC [4,5] and should have been revised in 1996. In accordance with the Council Directives, an extensive network of analytical residue laboratories has been created for the purpose of veterinary inspections. This network consists of a *hierarchical* system of so-called routine and/or field laboratories (RFLs), about 40 national reference laboratories (NRLs) and four communities reference laboratories (CRLs). An overview of this strategic system of laboratories is presented elsewhere [6].

In 1995, in co-operation with the four CRLs, the Commission started the complete legal and technical revision of the two criteria Decisions [4,5]. Due to the complex nature of the revision process, the concurrent revision of the underpinning Council Directive 86/469/EEC and, last but not least, the demand for a stronger participation of the NRLs in the process, in May 1998 the Commission designated a working group to draft new or revised criteria. This working group was chaired by François André (Nantes, France) and supervised by the Commission and the four CRLs.

The working group was also requested to take into account the developments and progress made in related fields globally [7], e.g. within the relevant Committees of the FAO/WHO Codex Alimentarius and the control of doping in sports.

During the discussions in the working group the concept of identification points (IPs) for setting up quality criteria for qualitative methods (or the qualitative part of quantitative methods) was introduced and accepted. The basic idea of IPs is that a laboratory is allowed to use any molecular spectrometric technique or combination of techniques in order to obtain the minimum number of IPs necessary for the proper identification of a component. The minimum number of points that must be obtained for group A (banned) compounds is set to four. This number corresponds to the classical four ions (in correct ratios) of electron ionisation (EI) mass spectrometry [4].

However, a laboratory is not restricted to four points and may identify a component using more IPs, provided that this procedure does not produce a higher false negative rate. For compounds with an established maximum residue limit (MRL) (group B), a minimum of three IPs are required for satisfactory confirmation of the compound's identity. In this paper, in which all members of the working group participated, some of the aspects and background of the use of IPs are presented.

2. Limitations of current criteria

The current European criteria [3] require that when gas chromatography coupled to low resolution mass spectrometry (GC–LRMS) is used as a confirmatory method, it is preferred that the intensities of at least four diagnostic ions are measured. If the compound does not yield four diagnostic ions with the method used, the identification of the analyte should be based on the results of at least two independent GC–LRMS methods with different derivatisation and/or ionisation techniques, each producing two or three diagnostic ions. The molecular ion should preferably be one of the selected diagnostic ions. The relative abundances of all diagnostic ions of the analyte should match those of the standard analyte, preferably within a margin of ± 10% (EI mode) or ± 20% (chemical ionisation (CI) mode).

The most important limitations of these rules are threefold: (1) the criteria are not applicable to all

compounds, (2) the criteria are ambiguous, (3) application of the criteria is limited to GC–MS.

First, not all group A compounds (e.g. some β-agonists) generate four suitable diagnostic ions. Depending on the structure of the molecules some analytes show only two or three diagnostic ions. In such cases much time is spent on testing alternative derivatives in order to obtain the necessary ions for proper identification. Even if a component shows four diagnostic ions at a relatively high concentration (> 2 μg/kg), the less abundant ions may disappear when the concentration of the analyte decreases, creating false negative results according to the criteria. This is easily demonstrated by using a series of spiked samples.

The most important factor is the interpretation of the tolerance of the peak intensity ratios. For example: some analysts have interpreted the tolerances of ion ratios as being absolute (± 10%). When considering a spectrum consisting of one major diagnostic ion and three minor ones and interpreting the tolerances as absolute, the smaller ions may vary widely (e.g. between 1 and 21%). These tolerances are so wide that the spectrum will easily match the criteria if the major peak is present and lower intensity 'peaks' result only from noise.

Mass spectrometric detection can be carried out by recording full mass spectra for example by MS^n techniques (e.g. in ion traps) or by selected ion monitoring (SIM) and selected reaction monitoring (SRM) (e.g. in quadrupoles). Other MS or MS^n techniques in combination with separation techniques (column liquid chromatography (LC) or GC) and ionisation modes (e.g. EI, CI, atmospheric pressure chemical ionisation, electrospray ionisation (ESI)) can be used as well. The criteria for mass spectrometry in 93/256/EEC are not really adequate given the technical advances that have occurred recently.

3. Some 'new' definitions influencing quality criteria and IPs

3.1. New definitions of analytical parameters

In the draft revision of 93/256/EEC (since 2000 referred to as Commission document SANCO/1805/2000), definitions which may influence the use of IPs are discussed briefly. For example, a method is only considered quantitative if criteria for accuracy (sum of trueness and precision) are fulfilled. It is also highlighted that the identification of compounds has to be completed before their quantification. The definitions of the analytical limits will now be based on the detection capability (CCβ: the smallest content of the analyte identified by a specified set of identification parameters (so-called 'identifiers'), IPs inclusive, that may be detected or quantified in a sample with an error probability of β (likelihood of a false negative decision; β-error ≤5%)) and on the decision limit (CCα: the limit at which the content of the analyte in a sample is truly violative with an error probability of α (likelihood of a false positive decision)), replacing the formerly used limits of detection, determination and quantification. Some terms have been changed for regulatory reasons, 'violative result' replacing 'positive result', and 'non-violative result' replacing 'negative result'. In addition, a new regulatory limit was established, the 'minimum required performance limit' (MRPL: the content at which the method will give reliable results in terms of violative and non-violative). This will harmonise the minimum performance characteristics which European residue control laboratories must achieve in their analytical methods for banned substances. This level has to be established for each analyte–matrix combination in accordance with Council Directive 96/23/EC by the Commission in close co-operation with the four CRLs and serves the harmonisation of the performance levels of those laboratories.

3.2. Ion recognition

Ions must be defined in full-scan or mass fragmentography (SIM).

If mass spectrometric determination is performed by recording full-scan spectra, the presence of all measured diagnostic ions with a relative intensity of more than 10% in the reference spectrum of the standard analyte is obligatory.

If mass spectrometric determination is performed by fragmentography, the molecular ion should preferably be one of the selected diagnostic ions. The selected diagnostic ions should not exclusively originate from the same part of the molecule. The signal-to-noise ratio for each diagnostic ion must be ≥ 3:1. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense ion or transition, must correspond to those of the standard analyte, either from calibration standards or from spiked samples, at compara-

Table 1
Maximum permitted tolerances for various relative ion intensities

Relative intensity[a]	GC–EI–MS[b]	Other techniques[c]
> 50%	± 10%	± 20%
> 20–50%	± 15%	± 25%
> 10–20%	± 20%	± 30%
≤ 10%	± 50%	± 50%

[a]Relative intensity (in % of base peak).
[b]Tolerance relative to relative intensity (in % of peak intensity).
[c]GC–CI–MS, GC–MS^n, LC–MS, LC–MS^n.

ble concentrations and measured under the same conditions, within the tolerances given in Table 1.

Whenever a background correction is carried out, this must be performed uniformly throughout the batch.

3.3. *Interpretation of mass spectral data*

Mass spectrometric methods are suitable for consideration as confirmatory and/or reference methods only following either an on-line or an off-line chromatographic separation.

If full-scan spectra are recorded in single MS, a minimum of four diagnostic ions must be present with a relative intensity of ≥ 10% of the base peak. The molecular ion should be included if it is present in the reference spectrum with a relative intensity of ≥ 10%. Computer-aided library searching may be used instead of visual comparison. In this case, the comparison of the mass spectral data in the test samples to that of the standard analyte must exceed a critical match factor (e.g. 900/1000 for a reversed fit search). This factor should be determined during the validation process for every analyte on the basis of spectra for which the criteria described below are fulfilled.

Table 2
Relationship between nature of MS information and IPs earned

MS technique	IPs earned per ion
Low resolution mass spectrometry (LR)	1.0
LR–MS^n precursor ion	1.0
LR–MS^n transition products	1.5
High resolution mass spectrometry (HR)	2.0
HR–MS^n precursor ion	2.0
HR–MS^n transition products	2.5

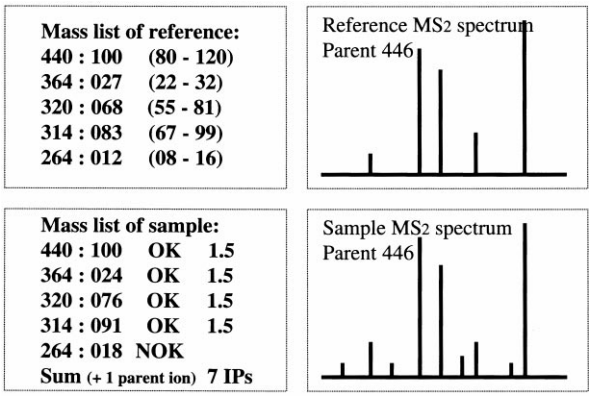

Fig. 1. Format to report IPs.

4. Quality criteria and IPs

If mass fragments are measured, a system of IPs shall be used to interpret the data. For the confirmation of group A and B substances, a minimum of four respectively three IPs is required. Table 2 shows the number of IPs that each of the basic mass spectrometric techniques can earn. However, in order to qualify for the IPs, a **minimum** of at least one ion ratio must be measured, **all measured ion ratios** must meet the criteria described above, and a **maximum** of three separate techniques can be combined to achieve the minimum number of IPs.

In determining the number of IPs the following remarks should be taken into account: of course each ion may only be counted once. GC–MS using EI is regarded as being a different technique to GC–MS using CI. Different chemical derivatives of an analyte can be used to increase the number of IPs only if derivatisation is based on different reaction chemistries (e.g. trimethylsilyl and heptafluorobutyryl derivatives). For the final confirmation of substances listed in group A, the selected methods must involve the use of mass spectrometry. However, since some analytes yield only three ions, the following techniques can be used to contribute a maximum of one IP: LC coupled with full-scan diode array spectrophotometry (DAD), LC coupled with fluorescence detection, LC coupled with an immunogram or two-dimensional TLC coupled with spectrometric detection, providing that the relevant criteria for these techniques (described in SANCO/1805/2000) are fulfilled.

In Table 3 some examples of the number of IPs that can be earned for a range of techniques and their combinations are given.

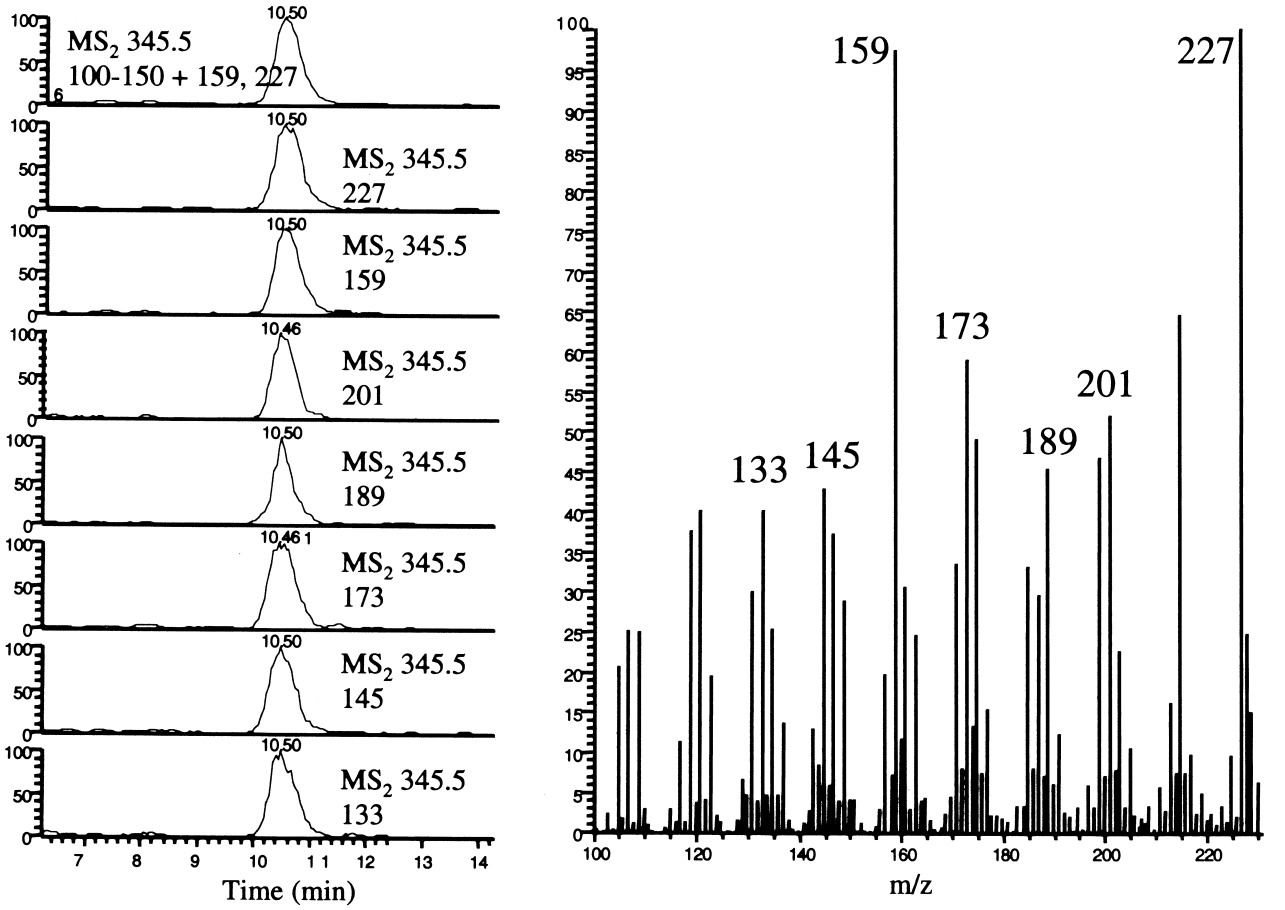

Fig. 2. LC–MS^2 chromatogram and spectra of a 'violative' sample (16β-hydroxystanozolol); precursor ion: m/z 345.5.

The practical application of IPs may be performed by computer programs. In Fig. 1 an example of a datasheet of a violative result is given.

5. Examples of the application of IPs

The following examples describe the application of the IP concept in residue analysis.

Table 3
Examples of number of IPs earned for a range of techniques and their combinations

Technique	Number of ions	IPs
GC–MS (EI or CI)	*n*	*n*
GC–MS (EI and CI)	2 (EI)+2 (CI)	4
GC–MS (2 derivs)	2 (derivative A)+2 (derivative B)	4
LC–MS	*n*	*n*
GC–MS–MS	1 precursor and 2 product ions	4
LC–MS–MS	1 precursor and 2 product ions	4
GC–MS–MS	2 precursor ions, each with 1 product	5
LC–MS–MS	2 precursor ions, each with 1 product	5
LC–MS–MS–MS	1 precursor, 1 product and 2 second transition products	5.5
HRMS	*N*	2*n*
GC–MS and LC–MS	2+2	4
GC–MS and GC–HRMS	2+2	4

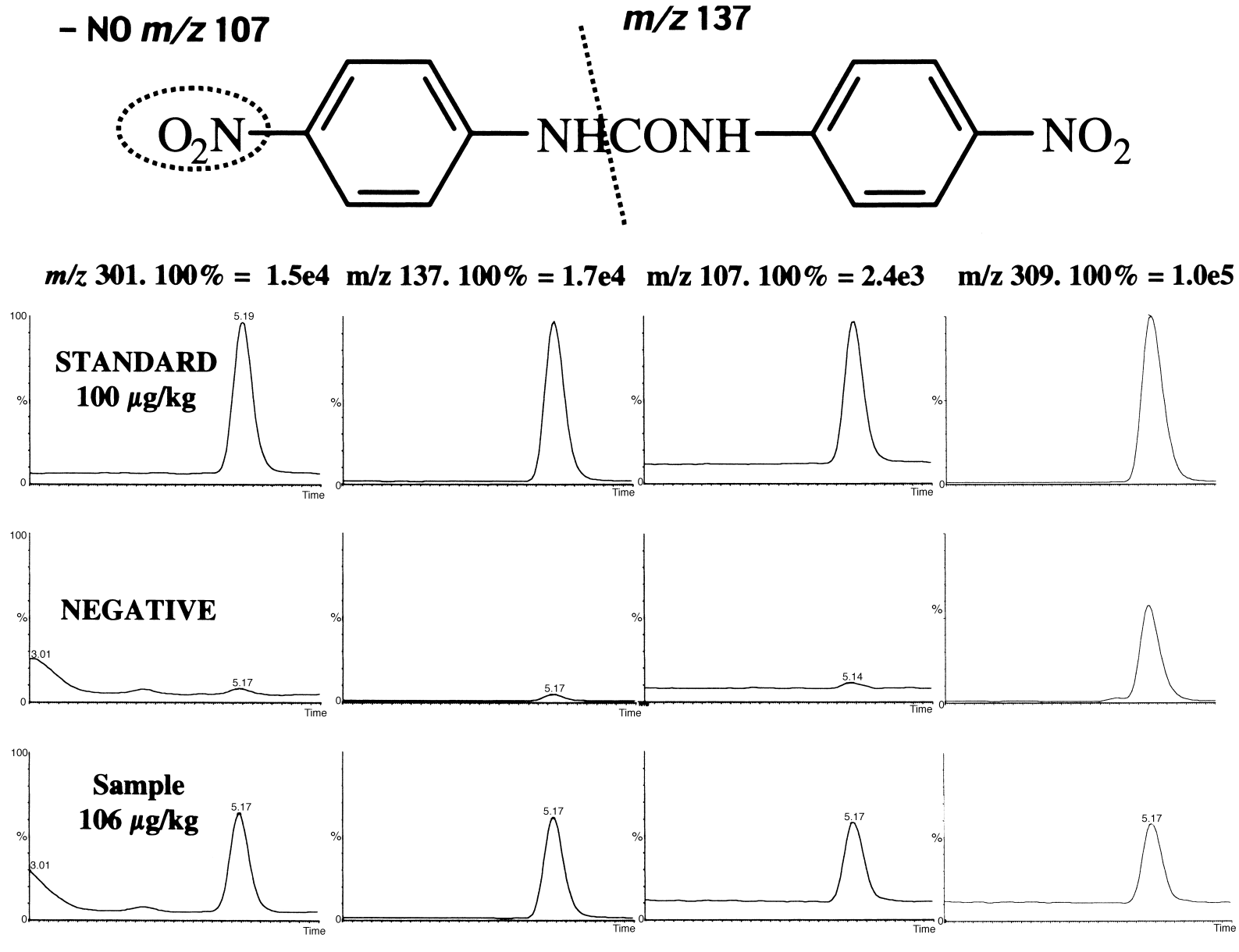

Fig. 3. LC–MS^2 chromatograms of nicarbazin (DNC) in standards and chicken liver.

5.1. Anabolic steroids: 16ß-hydroxystanozolol

The abuse of stanozolol (a group A component) in cattle fattening is detected by the determination of the major bovine metabolite (16β-hydroxystanozolol) by LC–MS^n [8,9]. Fig. 2 shows the chromatogram and spectrum of a 'violative' sample.

In Table 4 the calculation of IPs for 16β-hydroxystanozolol is presented. The parameters needed are: the relative intensities of ions to the base peak (m/z 159) in the MS^2 (product ion) spectrum of a spiked sample; the maximum permitted tolerances for relative ion intensities (calculated from Table 1). The relative intensities which match the tolerances are marked with a '#'. As can be seen in Table 4, the calculated number of IPs for the blank sample (non-violative sample) is one (there are no ions, except the base peak, which match the tolerances). For the violative samples 10 and 13 IPs are calculated, respectively. This number of IPs is clearly higher than the minimum number that has to be earned.

Table 4
Calculation of IPs for 16β-hydroxystanozolol[a]

m/z	Spike	Tolerance	Blank[b]	Sample 1[c]	Sample 2[d]
107	28	(21–35)	4	46	25#
121	37	(28–46)	1	40#	40#
133	49	(37–61)	2	40#	40#
145	40	(30–50)	/	36#	47#
159	**100**	(80–120)	7	**100#**	97#
173	61	(49–73)	4	75	59#
189	35	(26–44)	7	38#	45
201	46	(35–58)	1	51#	52#
227	88	(70–106)	**100#**	69	**100#**
	IPs	→	1	10	13

#Relative intensity within tolerance.
[a]Parameters needed: relative intensities of ions to base peak and tolerances in blank sample fortified with the analyte; relative intensities to base peak in a blank, a non-violative and two violative samples.
[b]Blank (non-violative sample).
[c]Sample 1: violative sample (low concentration).
[d]Sample 2: violative sample (higher concentration).

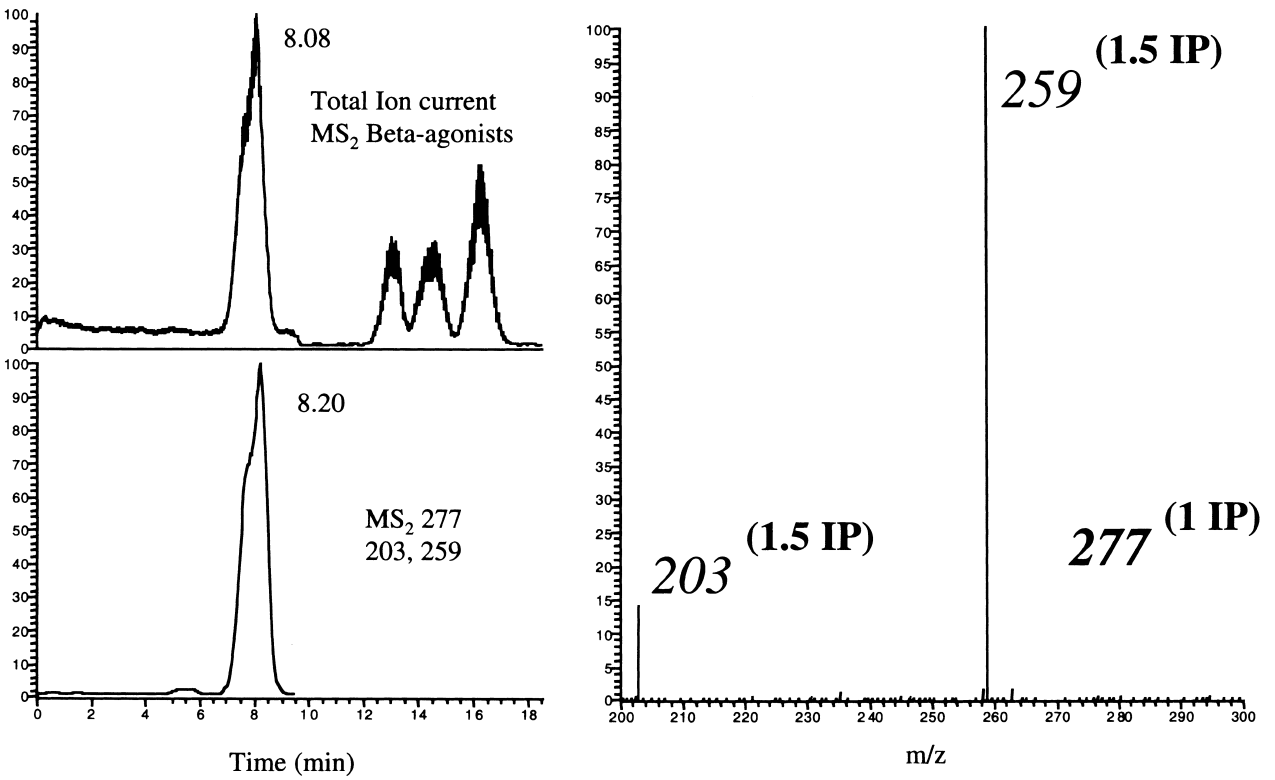

Fig. 4. LC–MS^2 chromatogram and spectra of a 'violative' sample (clenbuterol).

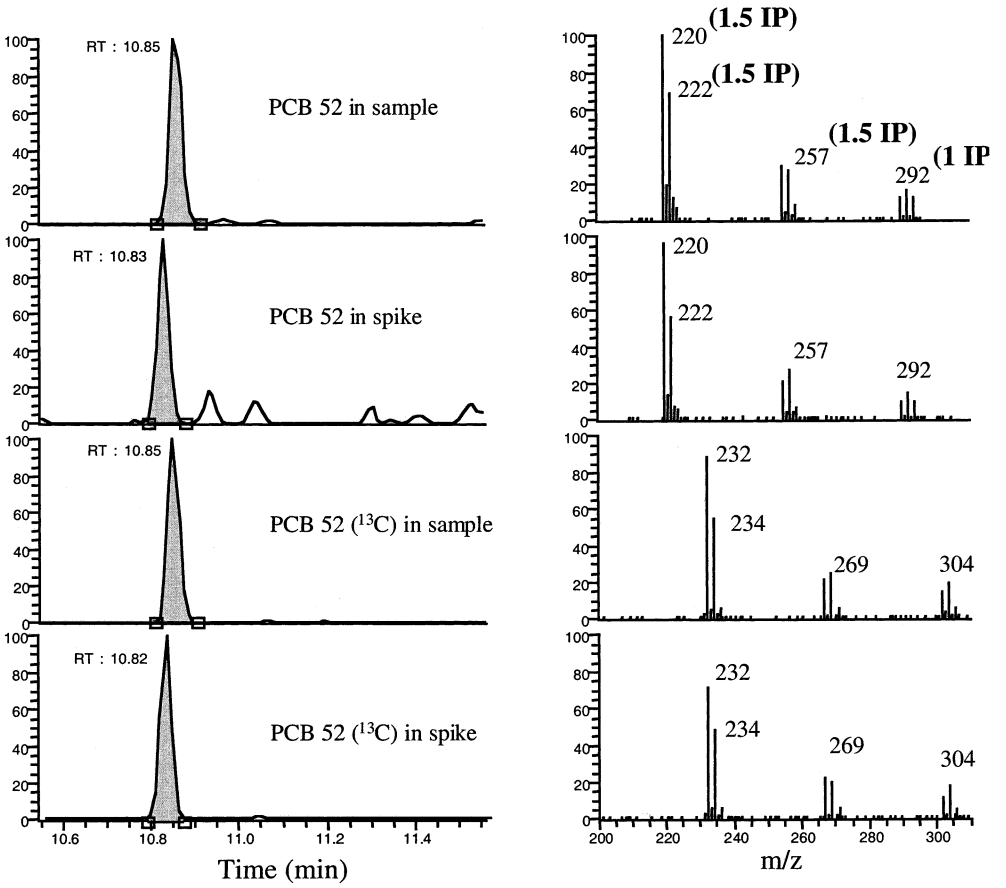

Fig. 5. GC–MS^2 chromatogram and spectra of PCB 52 in a fat sample compared to a spike and PCB 52 (^{13}C) internal standard.

5.2. β-Agonists: clenbuterol

Clenbuterol (a group A compound) is the most popular β-agonist in cattle fattening and is also used in sports doping. Fig. 3 shows an example of the determination of clenbuterol by LC–MS^2 [10]; only two diagnostic MS^2 ions are available. The minimum criteria for identification are reached by one precursor ion (m/z 277: 1 IP) and two product ions (at m/z 203 and 259: 3 IPs) which match the tolerances of the ion ratios calculated on the basis of a spiked sample.

5.3. MRL compound: nicarbazin

Nicarbazin, a coccidiostat, consists of 4,6-dimethyl-2-hydroxypyrimidine and 4,4′-dinitrocarbanilide (DNC), complexed in a 1:1 molar ratio. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has fixed an MRL of 200 μg/kg for DNC in the liver, kidney, muscle, fat or skin of broiler chickens. As a group B substance, three IPs are required for the confirmation of DNC. However, the LC–ESI MS–MS method employed [11] earns four IPs. In addition to the molecular ion $[M-H]^-$ at m/z 301 (1 IP), two transition ions at m/z 137 and 107 (3 IPs), and their corresponding ratios are monitored. These transitions correspond to scission (cleavage) of the molecule and loss of NO, as indicated in Fig. 4. This figure also shows SRM traces of a standard (100 μg/kg, equal to half the JECFA MRL), a negative liver sample and an incurred liver sample containing 106 μg/kg. Traces at m/z 309, corresponding to the internal standard d_8-DNC, are also shown.

5.4. MRL compound: avermectins and moxidectin

Avermectins and moxidectin are widely used in animal husbandry against nematode and arthropod parasites. A multi-residue method was developed and validated for the quantitation and confirmation

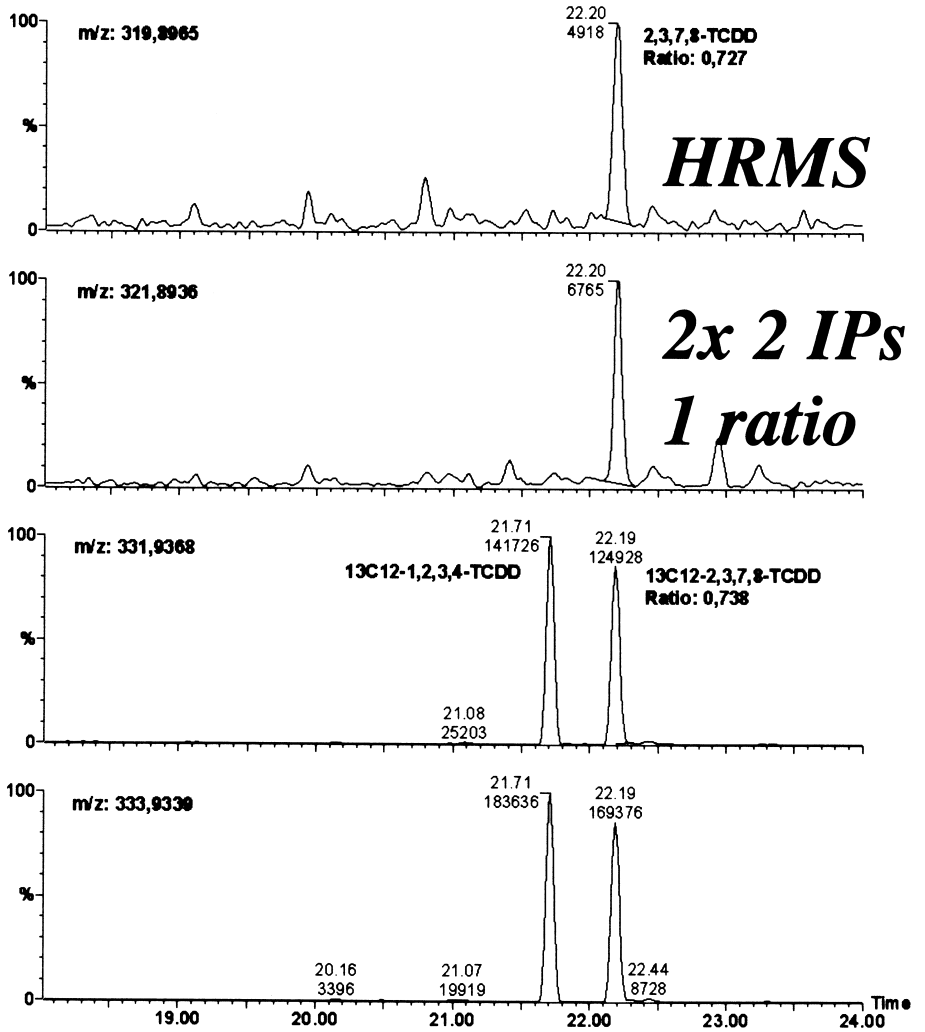

Fig. 6. HRGC–HRMS chromatograms of 2,3,7,8-TCDD in cow's milk.

of avermectins and moxidectin residues in bovine liver using (ion-trap) LC–MS^2 (Table 5) [12].

The high mass of the precursor and product ions reduces the probability that matrix components will produce isobaric interferences and the product ions selected should guarantee reliable confirmation as they derive from different parts of the parent molecule. The daughter ions were all detected with signal to noise ratios of $>3{:}1$ at concentrations at least an order of magnitude below their MRLs (10–

Table 5
Ions used for confirmation of the avermectins and moxidectin using LC–MS^2

Compound	Parent ion m/z $[M-H]^-$	Higher mass daughter ion	Lower mass daughter ion	Ratio of peak heights[a]
Eprinomectin	912.5	830.6	565.4	1.7 (29%)
Abamectin	871.5	789.6	565.4	2.1 (23%)
Doramectin	897.5	815.6	591.5	2.1 (19%)
Moxidectin	638.4	594.5	528.5	1.5 (24%)
Ivermectin	873.5	791.6	567.5	2.7 (17%)

[a]Values in parentheses are RSDs (n=144).

40 μg/kg), thus allowing a substantial margin for confirmation at the respective decision limits (CCα).

The higher mass product ion has less than 10% of the intensity of the precursor ion for all analytes and consequently a tolerance window of 50% was adopted. The ratios obtained with fortified and incurred samples were invariably within this tolerance when compared with standards of the same concentration analysed in the same batch. The method earns four IPs, exceeding the requirements for identification of licensed substances (three IPs).

5.5. Contaminants: polychlorobiphenyls (PCBs) and dioxins

PCBs are well-known contaminants that may enter the food chain. Their presence is monitored by the analysis of seven marker congeners in fat samples by GC–MS^2. Fig. 5 shows a chromatogram and MS^2 spectra of PCB 52 (one of the seven congeners) in a fat sample are given [13]. Identification is effected by one precursor ion (m/z 292: 1 IP) and three product ions with two ratios between them matching the tolerances (at m/z 220, 222 and 257: 4.5 IPs) yielding a total of 5.5 IPs.

Fig. 6 shows the determination of 2,3,7,8-TCDD in cow's milk at a concentration level of 0.30 pg/g fat.

The upper two fragmentograms show the traces for native TCDD and the lower two the traces for the ^{13}C-labelled internal standards. The analysis was performed HRGC/HRMS at a resolution of R_s=10 000. The target ratio for the two ions (319.8965 and 321.8936) monitored is 0.76. As can be seen all requirements (4 IPs from two HRMS ions) are fulfilled [14].

6. Conclusions

The use of IPs is a new approach to set up quality criteria for the identification of organic residues and contaminants. The system of IPs balances the identification power of the different analytical techniques and moreover has the advantage that new techniques may easily be incorporated in the procedure. The authors hope that this contribution may help in the general understanding, further testing and validating, and acceptance of this principle once the new criteria have been published.

The new criteria were discussed and adopted by the NRLs during a workshop in 1999 at Bilthoven (The Netherlands). The Commission document SANCO/1805/2000 was adopted by the CRLs in early 2000 and distributed in late 2000 to the EU Member States for legal comments. At present, the document – which may have far-reaching consequences for trade – is being circulated within the various involved Directorates General of the Commission for advice, consultation and approval.

Meanwhile many laboratories involved in the CRL–NRL–RFL network have already implemented the new criteria and in March 2000 the concept was also formally submitted by the Commission to the FAO/WHO Codex Alimentarius for consideration.

Acknowledgements

The authors wish to thank Ms Claire Gaudot (former DG VI) and Mr Jordi Serratosa (DG SANCO) for their interest in the revision of the criteria and their support of the working group.

References

[1] Council Directive 86/469/EEC of 16 September 1986 concerning the examination of animals and fresh meat for the presence of residues. Off. J. Eur. Communities L 275 (1986) 36.
[2] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decision 89/187/EEC and 91/664/EEC. Off. J. Eur. Communities L 125 (1996) 10.
[3] Commission Decision 89/610/EEC of 14 November 1989 laying down the reference methods and the list of national laboratories for detecting residues. Off. J. Eur. Communities L 351 (1989) 39.
[4] Commission Decision 93/256/EEC of 14 April 1993 laying down the methods to be used for detecting residues of substances having a hormonal or a thyreostatic action. Off. J. Eur. Communities L 118 (1993) 64.
[5] Commission Decision 93/257/EEC of 15 April 1993 laying down the reference methods and the list of national reference laboratories for detecting residues. Off. J. Eur. Communities L 118 (1993) 75.
[6] R.W. Stephany, J. Boiseau, B. Julicher, S. Caroli, in: N. Haagsma, A. Ruiter (Editors), Proc. EuroResidue III Conference, Veldhoven, 6–8 May, 1996, p. 149.
[7] R.W. Stephany, in: L.A. van Ginkel, A. Ruiter (Editors), Proc. EuroResidue IV Conference, Veldhoven, 8–10 May, 2000, p. 137.
[8] P. Delahaut, X. Taillieu, M. Dubois, K. De Wasch, H.F.

De Brabander, P. Batjoens, D. Courtheyn, Arch. Lebensmittelh. 49 (1998) 3.
[9] H.F. De Brabander, K. De Wasch, L.A. van Ginkel, S.S. Sterk, M.H. Blokland, P. Delahaut, X. Taillieu, M. Dubois, C.J.M. Arts, M.J. van Baak, L.G. Gramberg, R. Schilt, E.O. van Bennekom, D. Courtheyn, J. Vercammen, R.F. Witkamp, Analyst 123 (1998) 2599.
[10] K. De Wasch, H.F. De Brabander, D. Courtheyn, Analyst 123 (1998) 2701.
[11] S. Yakkundi, A. Cannavan, C.T. Elliott, T. Lövgren, D.G. Kennedy, Analyst (2000) submitted for publication.
[12] L. Howells, M.J. Sauer, in: L.A van Ginkel, A. Ruiter (Editors), Proc. Euroresidue IV Conference, Veldhoven, 8–10 May, 2000, p. 575.
[13] S. Impens, K. De Wasch, H.F. De Brabander, B. de Meulenaere, A. Huyghebaert, S. de Saeger, A. Heeremans, C. van Peteghem, M. Logghe, D. Courtheyn, R. van Renterghem, in: L.A. van Ginkel, A. Ruiter (Editors), Proc. EuroResidue IV Conference, Veldhoven, 8–10 May, 2000, p. 627.
[14] P. Furst, unpublished results.

François André is professor in the Ecole Nationale Vétérinaire Nantes, Laboratoire des dosages hormonaux, Nantes, France. The LDH is the National Reference Laboratory (NRL) in the framework of EU control of organic residues and contaminants.

Hubert De Brabander is professor of analytical chemistry of food in the Department of Veterinary Food Inspection, Laboratory of Chemical Analysis, Veterinary Faculty of Ghent University, Ghent, Belgium. Katia De Wasch and Sandra Impens are researchers in the same department.

Leen van Ginkel and Rainer Stephany are respectively the deputy director and the director of the European Union Community Reference Laboratory at the National Institute of Public Health and the Environment (RIVM) in Bilthoven, The Netherlands. Linda Stolker is a researcher in the same department. Rainer Stephany is also professor of analytical chemistry at the Department of the Science of Food of Animal Origin, Veterinary Faculty of Utrecht University, Utrecht, The Netherlands.

Robert Schilt is senior chemist and deputy study director and as such is responsible for the confirmatory analysis of growth promoting substances at the TNO Nutrition and Food Research in Zeist, The Netherlands.

Dirk Courtheyn is responsible for the analysis of growth promoting substances at the State Laboratory of the Ministry of Agriculture, Gentbrugge, Belgium.

Yves Bonnaire is director of the French National Laboratory for doping control on racehorses, France.

Peter Fürst is head of the Department for Central Analytical Services of the Chemical and Veterinary Control Laboratory in Münster, Germany.

Petra Gowik is the acting director of the Community Reference Laboratory and the National Reference Laboratory for residues of veterinary drugs at the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) in Berlin.

Glenn Kennedy is responsible for the confirmatory analysis of all classes of veterinary drug residues. He is employed in one of the UK National Reference Laboratories for veterinary drug residue analysis.

Thomas W. Kuhn is head of the section for growth promoting agents of the National Reference Laboratory at the Federal Veterinary Institute in Mödling, Austria.

Jean-Piere Moretain is scientific manager of the French National Reference Laboratory for veterinary drug residues, which is part of the Agency for Food Safety (AFSSA) and is located in Fougères, France.

Maurice Sauer is head of the Metabolism and Protein Binding Group, Department of Risk Research, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, UK.