Joint RIKILT-RIVM Report

RIKILT Report 87.27 1987-08-20
RIVM Report 368301 001

QUALITY CRITERIA FOR THE DETECTION OF ANALYTES IN TEST SAMPLES with special reference to anabolic agents and related compounds

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RIKILT Project 505 0060 Standardization Sampling and Analysis (Project leader: dr W.G. de Ruig)
The RIVM contribution to this report was performed within project 368301 (project leader: Dr. R.W. Stephany) on behalf and for the account of the Dutch Veterinary Chief Inspectorate of Public Health (VHI).

R8727

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SUMMARY

Analytical results serve a purpose and this can always be defined in terms of a decision to be taken. Examples are classifying a material according to value, or safety decisions such as "no action required, all is well". The nature of the decision determines the requirements put to the analysis such as the quantitative ones, e.g. limits of detection, or the qualitative ones, e.g. amount of certainty, that the identification of a compound is unambiguous. The classical approach to unambiguity is a detailed description of procedures.

However, this approach has some drawbacks. A standard method by virtue of its immobility is apt to become old-fashioned. Modern highly sophisticated apparatus ask for dedicated analytical procedures, which are not generally applicable. Changes in custom-made reagents or equipment force adaptation of the procedure.

For quantitative analysis the flexibility can be attained by a system of quality parameters. These are inadequate for qualitative analysis. In order to create a system in which well-defined limits of ambiguity can be set we have introduced the concept of "quality criteria" to be applied to the identification method(s) used.

The quality criteria for each detection method have to be stated and adopted by a board of experts for that detection method.

But, once an analysis fulfils the adopted criteria of a detection method, the presence of the investigated analyte in the sample can be taken as proved within the limits of ambiguity chosen for the decision process.

In particular for spectrometric identification methods such quality criteria are of essential interest.

Quality criteria are presented for low resolution MS, high resolution MS, IR, on line spectrometric detection with TLC and HPLC, furthermore for off line separation techniques (GC, TLC, HPLC), as well as general considerations for the whole analytical procedure.

The criteria are incorporated in the EEC "Commission Decision of 14 July 1987 laying down the methods to be used for detecting residues of substances having a hormonal action and substances having a thyrostatic action".

Throughout this report, some differences with respect to the EEC text occur.

1 LIST OF ABBREVIATIONS AND SYMBOLS

ISO = International Organization for Standardization

BCO = BCO Centre for Research, Breda, The Netherlands

CIVO = Central Institute for Food and Nutrition Research TNO, Zeist, The Netherlands

Janssen Pharmaceutica = Janssen Pharmaceutica, Beerse, Belgium

Organon= Organon International B.V., Oss, The Netherlands

RIKILT = State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands

RIVM = National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands

RUU = University of Utrecht, Utrecht, The Netherlands

General analytical

GC = gas liquid chromatography

HPLC = high pressure liquid chromatography

HPTLC = high performance thin layer chromatography

HRMS = high resolution mass spectroscopy

IA = immunoassay

IR = infrared spectroscopy

LRMS = low resolution mass spectroscopy

MS = mass spectroscopy

RIA = radioimmunoassay

Rf = relative retention distance

SOP = Standard Operating Procedure

SP = spectrometry, e.g. via diode array detection of UV range

TLC = thin layer chromatography

UV = ultra violet

/ = off-line hyphenated techniques

\- = on-line hyphenated techniques

e.g. HPLC/GC-MS = HPLC off line followed by GC with on-line MS.

For RIA:

T = total radioactivity (counts per minute, cpm or desintegrations per minute, dpm) added to a sample

B = radioactivity of the bound fraction of a sample

Bo = radioactivity of the bound fraction of a blank sample

B/Bo = fraction of the radioactivity of the bound fraction of a sample with respect to that of a blank sample ("fraction of binding with respect to zero binding")

%B/Bo = radioactivity of the bound fraction of a sample expressed as a percentage of the blank (i.e. B x 100/Bo)

Bo/T = fraction of the radioactivity of the bound fraction of a blank sample with respect to the added activity ("fraction of zero binding with respect to total")

NSB = non specific binding = aspecific binding (ASB)

For MS:

amu = atomic mass unit

CI = chemical ionization

EI = electron impact ionization

M = mass

Z = charge

HFB = heptafluorobutyric acid or heptafluorobutyryl derivative

MOX = methoxime derivative

TMS = trimethylsilyl derivative

MOX-TMS = methoxime and trimethylsilyl derivative

F^+ = fragment ion

$(F+1)^+$ = natural isotope satellite, 1 M/Z higher than the corresponding main isotope fragment ion

M^+ = molecular ion

$(M+1)^+$ = natural isotope satellite, 1 M/Z higher than the corresponding main isotope molecular ion.

2 INTRODUCTION

This document introduces a new approach to quality guarantees for analytical chemical investigations, viz. the application of well described quality criteria for interpretation of results instead of well described methods of analysis. The new approach holds for qualitative analysis, and aims to prevent false positive results.

In trade traffic, forensic control, and particularly in case of dispute, the results of chemical analysis have to be unambiguous and interpretable in only one way.

To achieve this certainty, it has become the usual practice to describe an analytical method in great detail. This is practised in Standard Methods of ISO and numerous international commodity organizations and national organizations for standardization.

Methods have to be described in terms of quality parameters such as specificity, accuracy, precision (repeatability and reproducibility), limit of detection, sensitivity, practicability and applicability.

To characterize the merits and the quality parameters of such a method, extensive investigations and collaborative studies have to be carried out.

The Codex Alimentarius Commission distinguishes four types of methods of analysis.

a) Defining Methods (Type I)

Definition: A method which determines a value that can only be arrived at in terms of the method per se and serves for calibration purposes.

Examples: Howard Mould Count, Reichert-Meissl value, loss on drying, salt in brine by density.

b) Reference Methods (Type II)

Definition: A type II method is the one designated Reference Method where type I methods do not apply. It should be selected from type III methods (as defined below). It should be recommended for use in cases of dispute and for calibration purposes.

Example: Potentiometric method for halides.

c) Alternative Approval Methods (Type III)

Definition: A type III method is one which meets the criteria required by the Codex Committee on Methods of Analysis and Sampling for methods that may be used for control, inspection or regulatory purposes.

Example: Volhard method or Mohr method for chlorides.

d) Tentative Method (Type IV)

Definition: A type IV method is a method which has been used traditionally or else has been recently introduced but for which the criteria required for acceptance by the Codex Committee on Methods of Analysis and Sampling have not yet been determined.

Examples: Chlorine by X-ray fluorescence, estimation of synthetic colours in foods.

It is clear that in the case of type I methods, where the result is defined by the method, an exact experimental protocol for the method is mandatory. However, the other types of method are also usually described in great detail. For these methods, however, the approach of exact formulation of the analytical procedure has some drawbacks, which are well recognized.

1. A standard method, by virtue of its immobility, may hamper new developments, and is apt to become old-fashioned.

2. Modern analytical procedures use frequently custom-made reagents and equipment, which can vary in chemical behaviour forcing adaptation of the procedure.

3. In modern analytical procedures, highly sophisticated and expensive apparatus and procedures are applied, which cannot be described in terms generally applicable in other laboratories.

A complication for qualitative analyses, in particular for residue analyses, is that some of the quality parameters as stated above, are irrelevant for qualitative results (e.g. repeatability and reproducibility). It is for this reason that there are so few standard qualitative methods. On the other hand, there is a need for unambiguous results in qualitative analysis as well.

To break this deadlock, "quality criteria" are introduced in this document persuing to prevent false positive results.
For a positive conclusion ("the analyte is identified in the sample examined"), the analytical results have to fulfil the quality criteria laid down for the detection method applied.

When consensus is achieved about the criteria, the conclusion holds, irrespective of the analytical procedure details leading to the analytical result.

Analytical procedures applying highly sophisticated techniques are not easily transferable to another laboratory in exactly the same way, making collaborative studies virtually impossible. Nevertheless the same quality criteria can be applied to the ultimate identification method.

For the application of adopted quality criteria for identification, collaborative studies are superfluous. Of course, collaborative studies remain useful to test the quality of a fully described analytical method, or to control the performance of laboratories.

The quality criteria for each identification method have to be stated and adopted by a board of experts for that identification method.

In this document, quality criteria are presented for radioimmunoassay, gas chromatography, thin layer chromatography (TLC), high pressure liquid chromatography (HPLC), HPLC and TLC with UV spectrum identification, gas chromatography with mass spectrometric identification and infrared spectroscopy, as well as general considerations for the whole analytical procedure.

The new approach, introduced in this document, does not imply that an exact experimental protocol ("Standard Operating Procedure", SOP) for a method of analysis should no longer be mandatory. In the framework of good laboratory practice each laboratory has the responsibility of making such protocol for its own methods of analysis, which are used in analytical control. The degree of specificity has to be known, and the result of the analysis should be related to this specificity. When the method is improved or altered, an adapted new protocol has to be made within the laboratory. SOP's should be available on request.

The criteria for mass spectrometry are extrapolated for general application from statements accepted by a working group of mass spectroscopie experts composed of R.W. Stephany (RIVM) (chairman), J. Freudenthal (RIVM), J.G. Leferink (BCO), M.C. ten Noever de Brauw (CIVO), A. Tas (CIVO) and L.G.M.Th. Tuinstra (RIKILT) on 1985-03-19. The criteria for infrared spectroscopy were stated by W.G. de Ruig (RIKILT) and J.M. Weseman (RIKILT) with advice from G. Dijkstra (RIVM). On 1987-03-19 the infrared criteria have been accepted as a reliable method for the detection of analytes in test samples by a group of experts on infrared spectroscopy composed of G. Dijkstra, (RIVM) (chairman), L.A. van Dijck (Organon), C. Funke (Organon), J.H. van der Maas (RUU), W.G. de Ruig (RIKILT), W.A. Seth Paul (Janssen Pharmaceutica) and R.W. Stephany (RIVM).

The criteria were submitted to the EEC working group "Methods of Analysis for Residues", consisting of W. Daelman, (EEC) (chairman), D. Arnold (FRG), Mrs. B. Boursier (France), N.F. Cunningham (UK), Mrs. G. Moretti (Italy), W.G. de Ruig (Netherlands) (rapporteur), R.W. Stephany (Netherlands) and H. Verburg (Netherlands), EEC doc VI/4705/86. This working group was in charge of formulating quality criteria for methods of analysis as required by EEC Council Directive of 1985-12-20 concerning the introduction of "Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption" (85/591/EEC). After some amendments, the criteria reported in this report were adopted by the Working Group, and combined with definitions and criteria collected by D. Arnold (Bundesgesundheitsamt, Berlin), EEC doc VI/4700/86. The latter were dealing with specificity, accuracy, precision, limit of detection, sensitivity, practicability and applicability, limit of decision, limit of quantification, calibration curves, interferences and reference material.

Both documents were brought together into a draft Commission Decision, EEC doc VI/6290/86.

On 1987-04-13, this draft was discussed and adopted with minor amendments by an ad hoc group of laboratory experts of all EEC member states.

On 1987-04-23, the Standing Veterinary Committee has unanimously adopted this draft, and eventually on 1987-07-14 the EEC Commission authorized it as "Commission Decision of 14 July 1987 laying down the methods to be used for detecting residues of substances having hormonal action and of substances having a thyrostatic action" (87/410/EEC, EEC Official Journal No L 223, 1987-08-11, p. 18-36).

Herewith the criteria stated in this report are incorporated in the above Commission Decision, with force of law in the European Community.

Summarizing:

Quality criteria are stated for qualitative analysis, to prevent false-positive results.

The criteria are applicable, irrespective of the applied method is ringtested or not.

The criteria present a high degree of flexibility as to the method applied.

3 DEFINITIONS REGARDING THE PRESENCE OF AN ANALYTE

3.1 Analyte: a component of a test sample the presence of which has to be demonstrated. The term "analyte" includes derivatives formed from the analyte during the analysis wherever this is applicable.

3.2 Standard material: a well defined substance in its highest attainable purity to be used as a reference in the analysis.

3.3 Positive result: the presence of the analyte in the sample is proved, according to the method, when the general criteria, and the criteria specified for the relevant detection method, are fulfilled.

3.4 Negative result: if not all of the general criteria, and the criteria specified for the relevant method are fulfilled, the analysis, according to the method, is not able to prove the presence of the analyte in the sample.

NOTE

The result of the analysis does not provide proof for the absence of the analyte in the sample.

3.5 Limit of decision: the lowest analyte content which, if actually present, will be detected with reasonable statistical certainly and can be identified according to the identification of the method.

3.5 Co-chromatography: the following procedure is applied.

The purified test solution prior to the chromatographic step is divided up into two parts.

a. One part is chromatographed as such.

b. The standard material of the analyte that is to be identified is added to the other part, and this mixed solution of analyte and standard material of analyte is chromatographed.

The amount of added standard material has to be about equal to the estimated or expected amount of the analyte.

4 GENERAL CONSIDERATIONS FOR THE WHOLE ANALYTICAL PROCEDURE

4.1 General criteria for the whole procedure

The method must have been proved to be able to distinguish between the analyte and all known interfering materials in the appropriate matrix.

The physical and chemical behaviour during the analysis of the analyte should be indistinguishable from that of the corresponding standard material in the appropriate matrix.

4.2 General criteria for separation techniques

4.2.1 Reference samples containing known amounts of analyte must be carried through the entire procedure simultaneously with each batch of test samples analysed. Alternatively, an internal standard may be added to test samples.

4.2.2 ~~*)~~ Appropriate reference samples having a content of standard material close to that of the expected analyte content of the samples must be subjected to the same derivatization procedure as the samples, including any post-derivatization clean-up.

4.3 Criterion for the off-line physical and/or chemical preconcentration, purification and separation, if applied

The analyte should be in the fraction that is characteristic for the corresponding standard material in the appropriate matrix material.

~~* Omitted from EEC Decision Commission~~

4.4 Criterion for on-line separation, if applied (e.g. GC)

The analyte should elute at the retention time which is characteristic for the corresponding standard material in the appropriate matrix material.

4.5 Preparation of the test sample

The test sample should be prepared from the laboratory sample in such a way that there is a maximum chance of detecting the analyte, if any present.

4.6 Preparation of the test portion

The test portion is prepared from the test sample in such a way that there is a maximum chance of detecting the analyte, if any present.

5 QUALITY REQUIREMENTS FOR DETERMINATION OF AN ANALYTE BY RIA

For screening purposes

5.1 The working range of the calibration curve has to be specified and has in general to cover a concentration range of at least one decade.

5.2 Control samples have to be included in each assay. Concentration levels: zero and at lower, middle and upper parts of the working range. Results for these have to be in line with those of previous assays.

5.3 At the limit of decision, the within-run coefficient of variation for the control samples has to be less than 0,15.

5.4 A minimum of 6 calibration points is required, adequately distributed along the calibration curve.

5.5 The recovery must be controlled and specified.

5.6 If logit-log transformation of the original data is applied, the within-run coefficient of correlation of the calibration curve has to be at least 0,985.

5.7 The calibration must have its highest precision around the limit of decision.

5.8 Adequate quality control parameters have to be in line with those of preceding assays, e.g. Bo/T, NSB, slope and intercept of the calibration curve.

NOTE Compliance with these quality requirements does not exclude the possibility of false positieve results orginating from systematic errors such as antibody cross reactivities and interference from non-representative sample material.

Reference: Nederlandse Staatscourant 250, December 23, 1983
= Benelux document SP/LAB/h (84) 17 (1984)
= EEC document VI/2447/84-EN

6 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY GC

6.1 The analyte should elute at the retention time which is characteristic for the corresponding standard material.

6.2 The nearest peak maximum in the chromatogram should be separated from the designated analyte peak by at least one full width at half maximum height.

6.3 For identification, additional co-chromatography in the GC stage is mandatory.

As a result, the peak presumed to be due to the analyte should be intensified only and the width at half maximum height should be within $\pm$ 10% of the original width. This requirement may be taken as fulfilled whenever the retention times are identical within 10% of the peak width at half maximum height.

Reference: RIVM report 368301 007 (1986)

7 CRITERIA FOR IDENTIFICATION FOR AN ANALYTE BY TLC OR HPTLC

7.1 The R_f value(s) of the analyte should agree with the R_f value(s) characteristic for the standard material. This requirement is fulfilled whenever the R_f value(s) of the analyte is (are) within 3% of the R_f value(s) of the standard material under the same conditions.

7.2 The visual appearance of the analyte should be indistinguishable form that of the standard material.

7.3 The centre of the nearest spot to that due to the analyte should be separated from it by at least half the sum of the spot diameters.

7.4 For identification, additional co-chromatography in the TLC step is mandatory. As a result the spot presumed to be due to the analyte should be intensified only; a new spot should not appear, and the visual appearance should not change.

7.5 For confirmation, two dimensional TLC is mandatory.

Reference: EEC document 690/VI/73, rev. version (1974)
Benelux document SP/LAB/h(74) 5 (1974)
Benelux document SP/LAB/h(76) 5 (1976)
RIVM Report 367910 004 (1983)
RIKILT SOPs A 441, A 443, A 444 (1986)

8 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY HPLC-SP

8.1 The maximum absorption wavelength in the spectrum of the analyte should be the same as that of the standard material within a margin determined by the resolution of the detection system. For diode array detection this is typically within $\pm$ 2 nm.

8.2 The spectrum of the analyte should not be visually different from the spectrum of the standard material for those parts of the two spectra with a relative absorbance > 10%. This criterion is met when the same maxima are present and at no observed point is the difference between the two spectra more than 10% of the absorbance of the standard material.

8.3 For identification, co-chromatography in the HPLC step is mandatory. As a result, the peak presumed to be due to the analyte should be intensified only.

Reference: RIVM report 368301 005 (1985)

9 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY TLC-SP OR HPTLC-SP

9.1 The R_f value(s) of the analyte should agree with the R_f value(s) characteristic for the standard material. This requirement is fulfilled when the R_f value(s) of the analyte is (are) within ± 3% of the R_f value(s) of the standard material under the same conditions.

9.2 The visual appearance of the analyte should be indistinguishable from that of the standard material.

9.3 The centre of the spot nearest to that due to the analyte should be separated from it by at least half the sum of the spot diameters.

9.4 For identification, additional co-chromatography in the TLC-step is mandatory. As a result the spot presumed to be due to the analyte should be intensified only; a new spot should not appear.

9.5 The maximum absorption wavelength in the spectrum of the analyte should be the same as that of the standard material, within a margin determined by the resolution of the detection system.

9.6 The spectrum of the analyte should not be visually different from the spectrum of the standard material.

10 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY GC-HRMS

10.1 To be classified as high resolution measurements the results should be obtained at a resolution of better than 9500 with a valley between adjacent peaks of no more than 10% of the peak heights.

10.2 The intensity ratio of the response of the analyte ions F^+ and $(F+1)^+$ or M^+ and $(M+1)^+$ should be equal to the theoretical value, within a margin of A %.

10.3 The fragment mass of the analyte (as determined by peak matching) should be equal to the theoretical value within a margin of B amu.

NOTE For a number of anabolic agents, values for A and B are listed in Table 1 of the Annex as examples. However, the method is not restricted to anabolic agents, but is generally applicable.

References:

Nederlandse Staatscourant 250, December 23, 1983 = Benelux document SP/LAB/h(84) 15 (1984)

RIVM Report U 3603/82 Endo(1982) = Benelux doc. SP/LAB/h (84) 22(1984)

RIVM Report U 3408/83 Endo(1983) = Benelux doc. SP/LAB/h (84) 21(1984)

RIVM Report U 206/84 Endo(1984) = Benelux doc. SP/LAB/h (84) 20(1984)

RIVM Report U 1598/84 Endo(1984)

11 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY GC-LRMS

11.1 Gas chromatographic criterion

11.1.1 The retention time of the analyte on GC must be the same as that of the standard material, within a margin of ± 5 seconds.

11.1.2 If an internal standard is used, then the relative retention time (B/A) of the analyte should be equal to that of the standard material within a margin of ± 5/A in the appropriate matrix, where:

A = the absolute retention time of an internal standard, in seconds

B = the absolute retention time of the analyte, in seconds

11.2 Mass spectrometric criteria

11.2.1 All ions monitored must be derived from analyte eluted at a single retention time.

11.2.2 The intensities of at least 4 (EI mode), or at least 2 and preferable more (CI mode), structure relevant ions must be determined.

11.2.3 The relative intensities of the ions detected, expressed as a percentage of the intensity of the ion of highest intensity (base peak) must be the same as those for the appropriate standard material, within a margin of ± 20 % (CI mode) or ± 10% (EI mode).

11.2.4 Mass spectrometric conditions should be optimized to obtain the molecular ion of the analyte in the MID spectrum, if possible at all.

NOTE In Tables 2-6 of the Appendix, details of the molecular and fragment ions of the TMS, MOX-TMS, MOX and HFB derivatives of a number of anabolic agents and related compounds are presented as examples. However, the criteria are not restricted to anabolic agents but are generally applicable.

11.2.5 Special criteria for dienoestrol, diethylstilboestrol and hexoestrol, after EI mode.

11.2.4.1 As the TMS derivatives of dienoestrol, diethylstilboestrol and hexoestrol produce only 3 fragment ions in the EI mode, the presence of these stilbenes can only be indicated.

11.2.4.2 The presence of dienoestrol and diethylstilboestrol is confirmed by analysing the HFB derivative, fulfilling the requirement in 11.2.2.

11.2.4.3 The presence of hexoestrol is indicated by the presence of the fragment ions (EI mode) 331, 332, 303 and 304 on analysing the HFB derivative.

For confirmation of the presence of hexoestrol, another aliquot is analysed under chemical ionisation conditions, in NH_3 gas, positive ions only.

The intensity ratio of the abundances of the analyte fragment ions of cluster 680 and 681 should be equal to that of the fragment ions of the standard material, within a margin of $\pm$ 10%.

References:

Nederlandse Staatscourant 250, December 23, 1983 = Benelux document SP/LAB/h(84) 16 (1984)

L.G.M.Th. Tuinstra, W.A. Traag, H.J. Keukens, R.J. van Mazijk: J. Chromatography 279 (1983) 533-542

RIKILT SOPs A 437, A 447, A 448, A 449 (1986)

12 CRITERIA FOR IDENTIFICATION OF AN ANALYTE BY IR SPECTROMETRY

12.1 Definition of adequate peaks

Adequate peaks are absorption maxima in the IR spectrum of a standard material, fulfilling the following requirements.

12.1.1 The absorption maximum is in the wavenumber range 1800-500 cm^{-1}.

12.1.2 The intensity of the absorption is not less than:

12.1.2.1 a specific molar absorbance coefficient of:

- 40 with respect to zero absorbance and
- 20 with respect to the peak base line

or

12.1.2.2 a relative absorbance of:

- 12,5% of the absorbance of the most intense peak in the region 1800-500 cm^{-1} when both are measured with respect to zero absorbance and
- 5% of the absorbance of the most intense peak in the region 1800-500 cm^{-1} when both are measured with respect to their peak base line.

NOTE Although adequate peaks according to 12.1.2.1 may be preferred from a theoretical point of view, those according to 12.1.2.2 are easier to determine in practice.

12.2 Number of adequate peaks

A minimum of 6 adequate peaks is required.

12.3 Coding of adequate peaks

The exact positions of the adequate peaks, in whole wavenumbers, act as objective, digitalized parameters for judging spectra of samples.

NOTE The Adequate Peaks in the IR spectra of 49 anabolic agents and related compounds are presented in Table 7 of the Appendix. However, the method is not restricted to anabolic agents, but is generally applicable.

12.4 Use of adequate peak tables.

12.4.1 The positions of the peaks in the IR spectrum of the analyte are compared with the positions of the adequate peaks in the IR spectrum of the standard material.

12.4.2 The number of peaks in the IR spectrum of the analyte whose frequencies correspond with an adequate peak in the IR spectrum of the standard material within a margin of $\pm$ 1 cm^{-1} is determined.

12.4.3 The "score" of the standard material in the analyte spectrum is calculated.

12.4.4 Definition of "score"

The "score" is the percentage of the adequate peaks of the standard material found in the IR spectrum of the analyte.

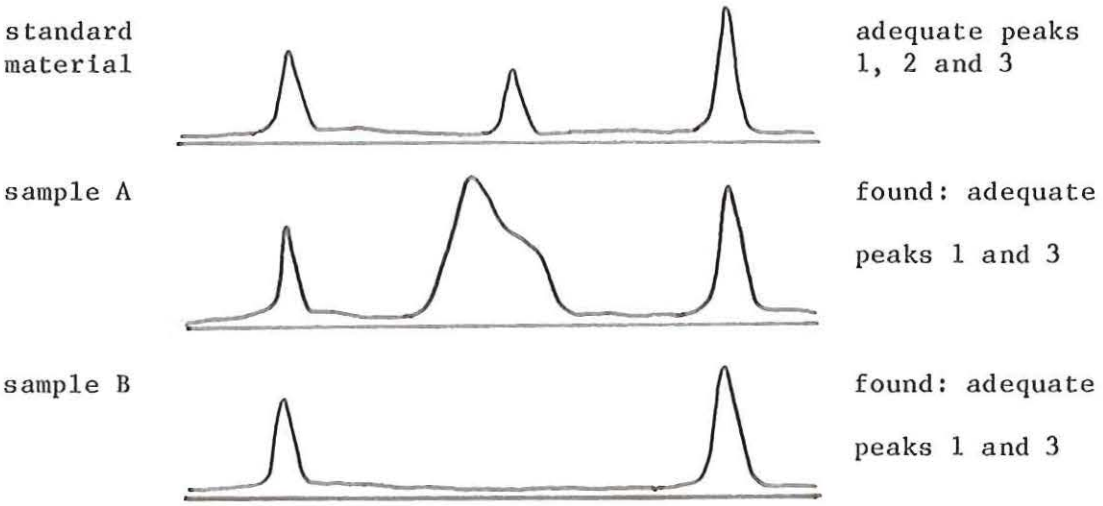
12.5 Criteria

12.5.1 The score shall be least 50%.

12.5.2 Where no match is found in the sample spectrum for an adequate peak of the standard material, the sample spectrum shall not exclude the absorption corresponding with the adequate peak (see figure 1).

12.5.3 The procedure is only applicable to absorption peaks in the sample spectrum with an intensity of at least 3 times the peak to peak noise.

Figure 1 Illustration of criterion 12.5.2



The spectrum of sample A does not exclude the presence of adequate peak 2 ——> criterion 12.5.2 fulfilled.

The spectrum of sample B excludes the presence of adequate peak 2 ——> criterion 12.5.2 is not fulfilled.

References:

W.G. de Ruig, J.M. Weseman. To be published.

RIKILT Reports 86.47, 86.48, 86.49, 86.87, 86.88, 86.89 (1986)

APPENDIX

Table 1: Data for confirmation of anabolics by HRMS

Derivative**	Ion mass		Intensity ratio		Ion mass	
	F^+ ion or M^+ ion M/Z value	$(F+1)^+$ ion or $(M+1)^+$ ion M/Z nominal value	theoretical value	A margin	M/Z theoretical value*	B M/Z
	amu	amu		%	amu	amu
DES-TMS	M^+ 412	413	0,3777	8	412,2254	0,0012
DE-TMS	M^+ 410	411	0,3772	8,5	410,2097	0,0012
HEX-TMS	F^+ 207	208	0,1889	17	207,1205	0,0007
NT-TMS	M^+ 346	347	not applied		346,2328	0,0015
NT-TMS	F^+ 256	257	not applied		256,1827	0,0015
NT-MOX-TMS	M^+ 375	376	not applied		375,2593	0,0015
epi NT-MOX-TMS	M^+ 375	376	not applied		375,2593	0,0015
E2-di-TMS	M^+ 416	417	not applied		416,2567	0,0015

* Calculated on the basis of ^{12}C isotope = 12,0000

** DES diethylstilboestrol
DE dienoestrol
HEX hexoestrol
NT 19-nortestosterone-17-β (nandrolone)
epiNT 19-nortestosterone-17-α
E2 oestradiol-17-β

Table 2: TMS derivatives of a number of anabolics and related compounds. Ions to be used for confirmation by LRMS (EI mode)

	MW	M/Z*			
Dienoestrol	410	410	395	381	
Diethylstilboestrol	412	412	397	383	
5α-Oestraan (3β,17α)diol	422	407	332	242	201
17α-Oestradiol	416	416	401	326	285
17β-Oestradiol	416	416	401	326	285
17β-Oestradiol-16,16,17(d3)	419	419	404	329	285
Ethinyloestradiol	440	440	425	300	285
Hexoestrol	414	207	191	179	
Methandrostenolone	372	372	357	302	282
Methyltestosterone	374	359	317	304	284
α-Nortestosterone	346	346	331	256	215
β-Nortestosterone	346	346	331	256	215
Testosterone	360	360	345	270	226
17-α-Trenbolone	342	342	252	237	211
17-β-Trenbolone	342	342	252	237	211
α-Zearalanol	538	538	523	433	307
β-Zearalanol	538	538	523	433	307
Zearalanone	464	464	449	335	307
Zearalenone	462	462	429	333	305

* Underlined ion: base peak
** Internal standard

Table 3: MOX-TMS derivatives of a number of anabolics and related compounds. Ions to be used for confirmation by LRMS (EI mode)

	MW	M/Z*			
Medroxyprogesteron	474	474	459	443	353
Methandrostenolone	401	401	386	370	280
Methyltestosterone	403	403	313	298	282
α-Nortestosterone	375	375	360	344	285
β-Nortestosterone	375	375	360	344	285
Testosterone	389	389	374	358	268
α-Trenbolone	371	371	281	266	253
β-Trenbolone	371	371	281	266	253
Zearalanone	493	493	478	462	406
Zearalenone	491	491	460	444	333

* Underlined ion: base peak

Table 4: MOX derivatives of a number of anabolics and related compounds. Ions to be used for confirmation by LRMS (EI mode)

	MW	M/Z*			
Medroxyprogesterone acetate	415	415	330	312	287
Megestrol acetate	413	413	353	338	310
Melengestrol acetate	425	425	365	350	322
Trenbolone acetate	341	341	298	281	266

* Underlined ion: base peak

Table 5: HFB-derivatives of a number of anabolics and related compounds. Ions to be used for confirmation by LRMS (EI mode)

	MW	M/Z*			
EI-mode					
Diethylstilboestrol	660	660	631	447	341
Dienoestrol (DE)	658	658	629	445	341
Hexoestrol (HEX)	662	332	331	304	303
17β-Oestradiol,16,16,17 (d3)**	667	667	454	412	359
CI-mode (NH_4 gas)					
Hexoestrol	662	681	680		

* Underlined ion: base peak
** Internal standard

Table 6: HFB derivatives of stilbenes. Ions to be used for confirmation by LRMS (CI mode).

a) Ammonia CI

	MW	M/Z*			
Hexoestrol	662	681	680	484	466
Diethylstilboestrol	660	678	482	<u>464</u>	

b) Methane CI

	MW	M/Z*			
Diethylstilboestrol	660	661	465	<u>464</u>	
Dienoestrol	658	659	463	462	<u>369</u>

* Underlined: base peak

Notes

1) Relative intensities of some of the above ions are too low (around 10 per cent) to be used reliably for confirmation.

2) Relative intensities of the ions may vary with amount of analyte injected onto the column. In the case of diethylstilboestrol at low concentrations under methane CI, the 661 ion becomes the base peak (i.e. there is less fragmentation to the mono-HFB form). It is therefore important to compare the relative ion intensities for the analyte with those for a standard material at about the same concentration.

3) Ionization with methane results in cleavage of the hexoestrol molecule. The fragment ions of lower mass are not well separated from co-extractives.

Table 7: Adequate Peaks in the infrared spectra of 49 anabolics and related products (cm^{-1}).

1 β E2	2 α E2	3 E2Ac	4 E2diAc	5 E2P	6 E2diP	7 E2S	8 E2Bz	9 E2ME	10 EE
		1701	1766 1734	1712	1763 1757 1733		1729		
1610	1610	1620		1696 1619			1600	1610	1615
1586	1586 1500	1584		1583				1577 1502	1584 1501
1498 1449 1416	1443	1498 1460	1494	1499 1460 1444	1492 1460 1419	1494 1420	1498 1451	1469 1444	1473 1449 1433
1382 1357 1320 1302	1379 1352	1373 1351	1375	1350	1381 1351	1392 1307	1380 1315	1374 1334 1313	1384 1358
1283 1250 1231	1284 1253 1234	1292 1276 1248 1235	1262 1248	1288 1249 1225 1212	1273 1247 1224	1241	1266 1223 1216	1291 1278 1252 1236	1299 1257 1203
1156 1130 1118 1102	1154 1119 1101	1152	1198 1177 1149	1151	1197 1154 1139	1176	1176 1152 1128	1183 1153 1130 1120	1184 1160 1147 1135 1122 1111
1056 1021 1012	1074 1054 1036 1013	1017	1040 1015	1086 1072 1012	1078 1055 1033 1014	1097 1075 1049 1018	1067 1025 1012	1055 1042 1025	1069 1055 1043 1022 1006
962 930 917 905	994 970 945 919		946	962	917	932 908			971 930 914
874 820	866 821	873	885	878 871 816	897 807	887 866 847 822 808	889	898 870 818	880 857 823
786 733	787					770	705	785	789
		624				650	688		646 622
	573					579 519			568

Table 7 Continued-1

11 M	12 E1	13 E3	14 Eq	15 Eqln	16 βT	17 αT	18 TAc	19 TP	20 TiC
	1719		1719	1717			1741	1729	1733
1612	1621	1610	1623	1622	1666 1658 1612	1654 1610	1672 1618	1669 1611	1667 1617
1580 1506	1584	1501	1588 1509 1500	1599					
1467 1449	1499	1452	1470 1407	1480 1460 1423	1470 1432	1432	1449 1433	1450	1470 1450
1377 1352 1325	1396 1361	1384 1353 1322	1354	1389	1378 1360	1380	1377 1362 1333	1331	1378 1331
1291 1255 1242	1287 1250	1285 1254 1238 1201	1278 1246	1225 1208	1277 1233	1276 1231	1274 1232	1270 1240	1294 1272 1230
1183 1165 1146 1133 1121 1109		1174 1149 1118 1103	1158 1148	1169	1199 1131 1114	1189		1185	1182 1125 1103
1062 1036 1019	1055	1068 1062 1034	1056	1066	1067 1056 1017		1041 1022	1080 1043 1020	1043 1009
967 905	920	964 943 928 917			957 943		945		941
862 844 833 823	877 819	886 871 851 818	875 810	849 817	870		863	863	864
789 702	788	787							
658 620	673	662							
		582							

Table 7 Continued-2

21 TD	22 TUn	23 TPP	24 TBz	25 MT	26 MT 9(11)	27 βNT	28 αNT	29 NTP	30 NTD
1734	1738	1734	1709					1741	1734
1670	1674	1672	1673	1664	1651	1666	1663	1650	1676
1618	1610	1615	1617	1611	1607	1619	1643	1620	1619
							1615		
1471	1473	1455	1452	1450		1412		1448	1465
1415		1424	1435					1422	1453
1379	1379	1381	1316	1374	1370	1335		1346	1381
1352	1335				1348				1332
1327	1315				1332				
1291	1276	1298	1275	1297	1274	1259	1258	1265	1258
1256	1249	1270	1229	1278	1232	1228	1211	1202	1212
1216	1208	1229		1234		1206			
1180	1172	1171	1178	1190	1188	1133	1135		1177
			1110	1156	1173				
					1153				
					1128				
					1104				
		1068	1070	1091		1075	1051	1085	
		1009	1023			1052		1052	
						1023		1025	
		944	998	950	956	967	968	968	
			941		937				
867	887	862	866	873	882	885	880	884	
		748	719						
		709							
		699	688		692				

Table 7 Continued-3

31 NTL	32 NTPP	33 Eti	34 βTB	35 αTB	36 TBA	37 P	38 MP	39 MPA	40 MGA
1735	1730				1737			1732	1731
								1717	1710
1676	1675	1659	1639	1643	1660	1699	1696	1673	1664
1619	1618	1612				1663	1664	1608	1629
						1616	1603		
	1501		1569	1578	1573				1584
1473	1447	1430	1438	1450	1439	1439	1448		1460
1418		1418		1435					1447
1381	1330	1383	1379	1391	1375	1386	1349	1365	1390
1335		1331		1369		1358			1366
			1346	1346		1328			
			1322	1321					
				1310					
1299	1297	1288	1288	1279	1245	1279	1271	1261	1269
1269	1259	1232	1267	1240		1237	1233	1253	1260
1240	1205		1226	1229		1228			1247
1205						1204			1224
1176	1177	1191	1199	1198		1162	1186	1187	1167
		1125	1101	1150			1123		1143
				1126					1127
									1109
1053	1080	1068	1075	1088	1096	948	1093	1080	1083
	1049	1060	1054	1028	1053			1056	1059
			1017	1011	1023				1014
	965			937	987			965	963
	879	869		852		871	871		878
	752	724		795					
	704			762					
		697							
							597		

Table 7 Continued-4

41 MLGA	42 DE	43 DES	44 HEX	45 DEdiAc	46 DESdiP	47 Z	48 Cort	49 HCort
1738				1757	1763			1714
1716					1754			
1666	1619	1609	1613			1644	1694	1644
1625	1608					1615	1648	1610
							1618	
1579	1591	1590	1598	1503	1504	1587		
	1513	1514	1516					
1444	1426	1462	1458		1459	1464	1447	1432
1416		1427	1440				1413	
1389	1333	1337		1368	1363	1381	1391	
1372						1353		
1318						1311		
1260	1247	1282	1218	1218	1210	1259	1269	1271
1245	1205	1247				1200		1237
1231		1204						
1205								
1123	1171	1173	1174	1196	1157	1167	1186	1133
	1102	1114	1107	1164			1159	1115
1037		1012		1019	1076	1096	1075	1047
					1019	1075	1064	1006
							1037	
972				910		989		942
953								900
930								
881	853	851	847	865	895	840	875	865
	834	831	830					
	826	805	804					
	775	721	716					
627	649	646	646	679				
612	620							
551	520	586	573					
			510					

Table 7 Continued-5

List of abbreviations

1	βE2	Oestradiol-17β
2	αE2	Oestradiol-17α
3	E2Ac	Oestradiol-17-acetate
4	E2diAc	Oestradiol diacetate
5	E2P	Oestradiol-17-propionate
6	E2diP	Oestradiol dipropionate
7	E2S	Oestradiol-3-sulphate
8	E2Bz	Oestradiol-3-benzoate
9	E2ME	Oestradiol-3-methylether
10	EE	Ethinyloestradiol
11	M	Mestranol
12	E1	Oestrone
13	E3	Oestriol
14	Eq	Equilin
15	Eqln	Equilenin
16	βT	Testosterone-17β
17	αT	Testosterone-17α
18	TAc	Testosterone acetate
19	TP	Testosterone propionate
20	TiC	Testosterone isocaproate
21	TD	Testosterone decanoate
22	TUn	Testosterone undecanoate
23	TPP	Testosterone phenylpropionate
24	TBz	Testosterone benzoate
25	MT	Methyltestosterone-17α
26	MT-9(11)	Methyltestosterone-Δ9(11)
27	βNT	Nortestosterone-17β
28	αNT	Nortestosterone-17α
29	NTP	Nortestosterone propionate
30	NTD	Nortestosterone decanoate
31	NTL	Nortestosterone laurate
32	NTPP	Nortestosterone phenylpropionate
33	Eti	Ethisterone
34	βTB	Trenbolone-17β
35	αTB	Trenbolone-17α
36	TBA	Trenbolone acetate
37	P	Progesterone
38	MP	Medroxyprogesterone
39	MPA	Medroxyprogesterone acetate
40	MGA	Megestrol acetate
41	MLGA	Melengestrol acetate
42	DE	Dienoestrol
43	DES	Diethylstilboestrol
44	HEX	Hexoestrol
45	DEdiAc	Dienoestrol diacetate
46	DESdiP	Diethylstilboestrol dipropionate
47	Z	Zeranol
48	Cort	Corticosterone
49	HCort	Hydrocortisone