II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DECISION

of 14 July 1987

laying down the methods to be used for detecting residues of substances having a hormonal action and of substances having a thyrostatic action

(87/410/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 85/358/EEC of 16 July 1985 supplementing Directive 81/602/EEC concerning the prohibition of certain substances having a hormonal action and of any substances having a thyrostatic action [1], as amended by Regulation (EEC) No 3768/85 [2], and in particular Article 5 thereof,

Whereas according to Article 5 (2) of Directive 85/358/EEC it is the responsibility of the Commission to determine, in accordance with the procedure laid down in Article 11 of that Directive, the methods of sample analysis to be used for detecting residues of substances having a hormonal action and of substances having a thyrostatic action;

Whereas Article 4 (1) (b) of Council Directive 64/433/EEC of 26 June 1964 on health problems affecting intra-Community trade in fresh meat [3], as last amended by Directive 86/587/EEC [4], and the second subparagraph of Article 11 (4) of Council Directive 85/397/EEC of 5 August 1985 on health and animal health problems affecting intra-Community trade in heat-treated milk [5], as amended by Regulation (EEC) No 3768/85, provide that examinations for residues are to be carried out in accordance with proven methods which are scientifically recognized, in particular those laid down in Community directives or other international standards;

Whereas the determination of methods of sample analysis includes definition of the analytical procedures to be followed, the rules to be observed when drawing samples and the criteria to be applied when carrying out the analyses;

Whereas the analytical procedures adopted must be sufficiently sensitive to detect the presence of residues of substances having a hormonal action and of substances having a thyrostatic action;

Whereas sampling is an essential part of the method of analysis; whereas rules for the drawing of samples should therefore be laid down;

Whereas on 20 December 1985 the Council adopted Directive 85/591/EEC concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption [6]; whereas for the purposes of this Decision account should be taken of the criteria set out in point 1 of the Annex to that Directive;

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

The analytical procedures authorized for detecting residues of substances having a hormonal action and of substances having a thyrostatic action shall be the following:

(1) OJ No L 191, 23. 7. 1985, p. 46.
(2) OJ No L 362, 31. 12. 1985, p. 8.
(3) OJ No 121, 29. 7. 1964, p. 2012/64.
(4) OJ No L 339, 2. 12. 1986, p. 26.
(5) OJ No L 226, 24. 8. 1985, p. 13.
(6) OJ No L 372, 31. 12. 1985, p. 50.

— Immunoassay (IA),
— Thin layer chromatography (TLC),
— High performance liquid chromatography (HPLC),
— Gas chromatography (GC),
— Mass spectrometry (MS),
— Spectrometry (SP).

Article 2

Samples shall be drawn in accordance with the following rules :

1. the size of the sample must be sufficient to allow adequate analysis and to allow a repeat analysis and confirmatory tests ;

2. the samples must be marked in such a way that identification remains possible at all stages of the procedure ;

3. the packaging, preservation and transport of the samples must be such as to maintain their integrity and not prejudice to result of the examination.

Article 3

The criteria to be applied when carrying out the analyses are set out in the Annex hereto.

Article 4

This Decision shall be re-examined before 1 January 1991 in order to take account of the evolution of scientific and technical knowledge.

Article 5

This Decision is addressed to the Member States.

Done at Brussels, 14 July 1987.

For the Commission
Frans ANDRIESSEN
Vice-President

ANNEX

CHAPTER I : GENERAL CRITERIA

Introduction

For the objective evaluation of residue-detection procedures, quantitative data on some standard characteristics of the procedures must be available. In addition, quantitative requirements must be laid down for certain of these characteristics which take into account, *inter-alia*, the purpose of the method (e.g. screening, quantitative determination, confirmation). In this chapter the criteria laid down in the Annex to Directive 85/591/EEC are defined as they shall be applied to the examination of routine methods for the analysis of residues of substances having a hormonal action and of substances having a thyrostatic action.

Definitions

(i) *Specificity*

Specificity is the ability of a method to distinguish between the analyte being measured and other substances. This characteristic is predominantly a function of the measuring principle used. Details concerning specificity must relate at least to any substances which might be expected to give rise to a signal when the measuring principle described is used, e.g. homologues, analogues, metabolic products of the residue of interest. From the details concerning specificity, it must be possible to derive quantitatively the extent to which the method can distinguish between the analyte and the other substances under the experimental conditions.

(ii) *Accuracy*

Accuracy in this document refers to accuracy of the mean. The definition which shall be used here is laid down in ISO 3534-1977 under 2.83 (Accuracy of the mean : the closeness of agreement between the true value and the mean result which would be obtained by applying the experimental procedure a very large number of times).

Note : The principal limitations on accuracy are :

(a) random errors ;

(b) systematic errors, e.g. low recovery.

For a very large number of experiments, the accuracy of the mean approaches the systematic error.

For desk review of a method, the number of experiments must be specified.

The measure of accuracy to be used is the difference between the mean measured value for reference material and the true value, expressed as a percentage of the true value.

(iii) *Precision ;* repeatability intra-laboratory (within laboratory) and reproducibility inter-laboratory (within and between laboratories) variabilities.

The general statistical term 'precision' shall be used as defined in ISO 3534-1977, 2.84 (Precision : the closeness of agreement between the results obtained by applying the experimental procedure several times under prescribed conditions).

According to the Annex to Directive 85/591/EEC, the precision values for methods of analysis which are to be considered for adoption under the provisions of that Directive shall be obtained from a collaborative trial which has preferably been conducted in accordance with ISO 5725-1986. For this purpose, the terms repeatability and reproducibility are defined in ISO 5725-1986.

For the purpose of preselection of candidate methods by desk review, it is sufficient that data on repeatability are available. For this purpose, the term repeatability is used here as defined in ISO 3534-1977 under 2.85 (a) (Repeatability : the closeness of agreement between successive results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory and short intervals of time)).

The measure of repeatability to be used is the coefficient of variation as defined in ISO 3534-1977, 2.35 (Coefficient of variation : the ratio of the standard deviation to the absolute value of the arithmetic mean).

(iv) *Limit of detection*

The limit of detection is the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. It is equal to the mean of the measured content of representative blank samples ($n \geq 20$) plus three times the standard deviation of the mean.

Note: If it is to be expected that factors such as species, sex, age, etc. may influence the characteristics of the method, then a set of blank samples is required for each individual homogeneous population to which the method is to be applied.

(v) *Sensitivity*

Sensitivity is a measure of the ability of a method to discriminate between small differences in analyte content. In this document, sensitivity is defined as the slope of the calibration curve at the level of interest.

(vi) *Practicability and applicability*

Practicability is a non-standard characteristic of an analytical procedure. It is dependent on the scope of the method and is determined by requirements such as sample throughput and costs.

Applicability is a list of the commodities to which the candidate method can be applied as presented or with minor modifications.

(vii) *Other criteria which may be selected as required*

Limit of decision

The limit of decision is the lowest analyte content which, if actually present, will be detected with reasonable statistical certainty and can be identified according to the identification criteria of the method. If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of decision is equal to the mean of the measured content of the representative blank samples plus six times the standard deviation of the mean.

Limit of quantification

The limit of quantification is the smallest measured content above which a determination of the analyte is possible with a specified degree of accuracy and repeatability (within laboratory).

Requirements as regards accuracy and repeatability

Accuracy:

In the case of repeated analysis of the reference sample, the deviation of the mean from the true value, expressed as a percentage of the true value, shall not lie outside the following limits:

	limits
— true value up to 1 µg/kg:	—50 % to +20 %
— true value over 1 µg/kg and up to 10 µg/kg:	—30 % to +10 %
— true value over 10 µg/kg:	—20 % to +10 %

Repeatability:

In the case of repeated analysis of the reference sample, the coefficient of variation (C.V.) of the mean shall not exceed the following values:

	C.V.
— mean up to 1 µg/kg:	0,30
— mean over 1 µg/kg and up to 10 µg/kg:	0,20
— mean over 10 µg/kg:	0,15

Calibration curves

If the method depends on calibration curves, then the following information must be given:

— the mathematical formula which describes the calibration curve,

— numerical values of the parameters of the calibration curves with 95 % confidence intervals (under optimal conditions),

— acceptable ranges within which the parameters of the calibration curve may vary from day to day,

— the working range of the calibration curve,

— details of the variance of the variables which is valid at least for the working range of the calibration curve.

Susceptibility to interference

For all experimental conditions which could in practice be subject to fluctuation (e.g. stability of reagents, composition of the sample, pH, temperature) any variations which could affect the analytical results shall be indicated.

If it is known that certain substances can interfere with the determination of the analyte, this shall also be indicated, together with any suggested means of overcoming the problem. It is of prime importance that any interference which might arise from matrix components should be investigated. Therefore, at least the largest sample equivalent which has no interfering effect on the determination of the analyte (after any specified sample 'clean-up') shall be indicated.

Relationship between tolerance values and analytical limits

For substances with a zero tolerance, the limit of decision of the analytical method must be sufficiently low that residue levels which would generally be expected after illegal use will be detected with at least 95 % probability.

For substances with a tolerance level, established at the maximum natural physiological level, the limit of quantification shall not exceed that tolerance minus three times the standard deviation which the method produces for a sample at the tolerance level.

Reference material

A reference material is a sample of substance or a single manufactured object of which one, or several, properties are determined with sufficient accuracy, so that it can be used to calibrate an apparatus or to verify a method of measurement. The certification must be based on a technically valid procedure.

If no reference material is available, relevant parameters may be evaluated by analysing fortified sample material.

CHAPTER II : QUALITY CRITERIA FOR THE INTERPRETATION OF RESULTS

1. List of abbreviations and symbols

General analytical

GC	= gas chromatography ;
HPLC	= high pressure liquid chromatography ;
HPTLC	= high performance thin-layer chromatography ;
HRMS	= high resolution mass spectrometry ;
IA	= immunoassay ;
IR	= infrared ;
UV	= ultraviolet ;
LRMS	= low resolution mass spectrometry ;
MS	= mass spectrometry ;
RIA	= radioimmunoassay ;
SP	= spectrometry, e.g. diode array ;
TLC	= thin-layer chromatography ;
/	= off-line hyphenated techniques ;
—	= on-line hyphenated techniques ;

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

For RTA :

cpm	= counts per minute ;
dpm	= disintegrations per minute ;
T	= total radioactivity (cpm or 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 ;
Bo/T	= fraction of the radioactivity of the bound fraction of a blank 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 ;
MID	= multiple ion detection ;
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

The quality criteria for the interpretation of results obtained with analytical methods shall be applied by laboratories carrying out tests for the detection of residues of substances having a hormonal action and of substances having a thyrostatic action, in accordance with the requirements of this chapter. The quality criteria are designed for qualitative analysis and aim to prevent false positive results. For a positive conclusion, the analytical results have to fulfil the quality criteria laid down for the detection 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:* 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. The result of the analysis is 'positive'.

3.4. *Negative:* the result of the analysis is regarded as 'negative' if the criteria specified for the relevant method are not fulfilled or the analysis does not indicate the presence of the analyte in the sample above the limit of decision.

3.5. *Co-chromatography:* the following procedure is applied. The purified test solution prior to the chromatographic step is divided into two parts:

(a) one part is chromatographed as such;

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

The amount of added standard material has to be about equal to the estimated 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 chemikl 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*

Reference samples containing known amounts of analytes 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.

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

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

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 present.

4.6. *Preparation of the test portion*

The test portion would be prepared from the test sample in such a way that there is a maximum chance of detecting the analyte, if 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 coefficient of variation for the control samples has to be less than 0,15.

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

5.5. The recovery must be controlled and specified.

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

5.7. 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 positive results originating from systematic errors such as antibody cross reactivities and from interference from non-representative sample material.

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 ± 10 % of the original width. This requirement may be taken as having been fulfilled when the retention times are identical within 10 % of the peak width at half maximum height.

7. **Criteria for identification of 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 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.

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

7.3. 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.

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.

8. **Criteria for identification of an analyte by HPLC-SP**

8.1. The maximum absorption wavelength in the UV 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 ± 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 itensified only.

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 better than 9 500 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 ions F^+ and $(F+1)^+$ or M^+ and $(M+1)^+$ of the standard material derivative should be equal to the theoretical value, within a margin of A %.

10.3. The fragment mass of the analyte derivation (as determined by peak matching) should be equal to the theoretical value of the corresponding derivative of the reference material, 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 Appendix as examples. However, the method is not restricted to anabolic agents, but is generally applicable.

11. **Criteria for identification of an analyte by GC-LRMS**

11.1. *Gas chromatographic criteria*

11.1.1. The retention time of the analyte on GC must be the same as that of the reference standards, 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 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 two, and preferably more, ions must be determined.

Note: For unambiguous identification of an analyte, an adequate number of ions must be selected, the number dependent on the particular compound and the specificity of the ions chosen. For the majority of the derivatives of anabolic substances listed in Tables 2 to 6 of the Appendix, selection of at least four ions is recommended.

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 reference standard within a margin of ± 20 % (CI mode) or ± 10 % (EI mode).

11.2.4. The molecular ion of the standard material should, if possible, be present in the MID spectrum of the analyte.

Note: In Tables 2 to 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 these derivatives but are generally applicable.

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 1 800-500 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 1 800-500 cm^{-1} when both are measured with respect to zero absorbance, and

— 5 % of the absorbance of the most intense peak in the region 1 800-500 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 12.12.2 are easier to determine in practice.

12.2. *Number of adequate peaks*

A minimum of six 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: Adequate peaks in the IR spectra of 49 anabolic agents and related compounds are presented in Table 7 of 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 found in the IR spectrum of the analyte.

12.5. *Criteria*

12.5.1. The score shall be at least 50 %.

12.5.2. The procedure is only applicable to absorption peaks in the sample spectrum with an intensity of at last three times the peak-to-peak noise.

APPENDIX

TABLE 1

Data for confirmation of anabolics by HRMS

Derivative (1)	Ion mass		Intensity ratio		Ion mass	
	F^+ ion or M^+ ion, M/Z nominal value (amu)	$(F+1)^+$ ion or $(M+1)^+$ ion, M/Z nominal value (amu)	Theoretical value	A margin (%)	M/Z theoretical value (2) (amu)	B M/Z margin (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

(1) DES = diethylstilboestrol,
DE = dienoestrol,
HEX = hexoestrol,
NT = 19-nortestosterone-17β (nandrolone),
epiNT = 19-nortestosterone-17α,
E2 = oestradiol-17-β.

(2) Calculated on the basis of ^{12}C = 12,0000.

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 (1)			
Dienoestrol	410	410	395	381	
Diethylstilboestrol	412	412	397	383	
5α-Oestrane-(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) (2)	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

(1) Underlined ion : base peak.
(2) 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 (1)			
Medroxyprogesterone	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

(1) 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 (1)			
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

(1) 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 (1)			
Diethylstilboestrol	660	660	631	447	341
Dienoestrol	658	658	629	445	341
Hexoestrol	662	332	331	304	303
17β-Oestradiol,16,16,17(d3) (2)	667	667	454	412	359

(1) Underlined ion : base peak.
(2) Internal standard.

TABLE 6

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

(a) *Ammonia CI*

	MW	M/Z (1)			
Hexoestrol	662	681	680	484	466
Diethylstilboestrol	660	678	482	464	

(1) Underlined ion : base peak.

(b) *Methane CI*

	MW	M/Z (1)			
Diethylstilboestrol	660	661	465	464	
Dienoestrol	658	659	463	462	369

(1) Underlined ion : base peak.

Notes

1. Relative intensities of some of the above ions are too low (around 10 %) 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 reference standard 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 IR spectra of 49 anabolics and related compounds

1	2	3	4	5	6	7	8	9	10
βE2	αE2	E2Ac	E2diAc	E2P	E2diP	E2S	E2Bz	E2ME	EE
		1 701	1 766 1 734	1 712	1 763 1 757 1 733		1 729		
1 610	1 610	1 620		1 696 1 619			1 600	1 610	1 615
1 586	1 586 1 500	1 584		1 583				1 577 1 502	1 584 1 501
1 498 1 449 1 416	1 443	1 498 1 460	1 494	1 499 1 460 1 444	1 492 1 460 1 419	1 494 1 420	1 498 1 451	1 469 1 444	1 473 1 449 1 433
1 382 1 357 1 320 1 302	1 379 1 352	1 373 1 351	1 375	1 350	1 381 1 351	1 392 1 307	1 380 1 315	1 374 1 334 1 313	1 384 1 358
1 283 1 250 1 231	1 284 1 253 1 234	1 292 1 276 1 248 1 235	1 262 1 248	1 288 1 249 1 225 1 212	1 273 1 247 1 224	1 241	1 266 1 223 1 216	1 291 1 278 1 252 1 236	1 299 1 257 1 203
1 156 1 130 1 118 1 102	1 154 1 119 1 101	1 152	1 198 1 177 1 149	1 151	1 197 1 154 1 139	1 176	1 176 1 152 1 128	1 183 1 153 1 130 1 120	1 184 1 160 1 147 1 135 1 122 1 111
1 056 1 021 1 012	1 074 1 054 1 036 1 013	1 017	1 040 1 015	1 086 1 072 1 012	1 078 1 055 1 033 1 014	1 097 1 075 1 049 1 018	1 067 1 025 1 012	1 055 1 042 1 025	1 069 1 055 1 043 1 022 1 006
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

11	12	13	14	15	16	17	18	19	20
M	E1	E3	Eq	Eqln	βT	αT	TAc	TP	TiC
	1 719		1 719	1 717			1 741	1 729	1 733
1 612	1 621	1 610	1 623	1 622	1 666 1 658 1 612	1 654 1 610	1 672 1 618	1 669 1 611	1 667 1 617
1 580 1 506	1 584	1 501	1 588 1 509 1 500	1 599					
1 467 1 449	1 499	1 452	1 470 1 407	1 480 1 460 1 423	1 470 1 432	1 432	1 449 1 433	1 450	1 470 1 450
1 377 1 352 1 325	1 396 1 361	1 384 1 353 1 322	1 354	1 389	1 378 1 360	1 380	1 377 1 362 1 333	1 331	1 378 1 331
1 291 1 255 1 242	1 287 1 250	1 285 1 254 1 238 1 201	1 278 1 246	1 225 1 208	1 277 1 233	1 276 1 231	1 274 1 232	1 270 1 240	1 294 1 272 1 230
1 183 1 165 1 146 1 133 1 121 1 109		1 174 1 149 1 118 1 103	1 158 1 148	1 169	1 199 1 131 1 114	1 189		1 185	1 182 1 125 1 103
1 062 1 036 1 019	1 055	1 068 1 062 1 034	1 056	1 066	1 067 1 056 1 017		1 041 1 022	1 080 1 043 1 020	1 043 1 009
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							

21	22	23	24	25	26	27	28	29	30
TD	TUn	TPP	TBz	MT	MT-D9 (11)	βNT	αNT	NTP	NTD
1 734	1 738	1 734	1 709					1 741	1 734
1 670 1 618	1 674 1 610	1 672 1 615	1 673 1 617	1 664 1 611	1 651 1 607	1 666 1 619	1 663 1 643 1 615	1 650 1 620	1 676 1 619
1 471 1 415	1 473	1 455 1 424	1 452 1 435	1 450		1 412		1 448 1 422	1 465 1 453
1 379 1 352 1 327	1 379 1 335 1 315	1 381	1 316	1 374	1 370 1 348 1 332	1 335		1 346	1 381 1 332
1 291 1 256 1 216	1 276 1 249 1 208	1 298 1 270 1 229	1 275 1 229	1 297 1 278 1 234	1 274 1 232	1 259 1 228 1 206	1 258 1 211	1 265 1 202	1 258 1 212
1 180	1 172	1 171	1 178 1 110	1 190 1 156	1 188 1 173 1 153 1 128 1 104	1 133	1 135		1 177
		1 068 1 009	1 070 1 023	1 091		1 075 1 052 1 023	1 051	1 085 1 052 1 025	
		944	998 941	950	956 937	967	968	968	
867	887	862	866	873	882	885	880	884	
		748 709	719						
		699	688		692				

31	32	33	34	35	36	37	38	39	40
NTL	NTPP	Eti	βTB	αTB	TBA	P	MP	MPA	MGA
1 735	1 730				1 737			1 732 1 717	1 731 1 710
1 676 1 619	1 675 1 618	1 659 1 612	1 639	1 643	1 660	1 699 1 663 1 616	1 696 1 664 1 603	1 673 1 608	1 664 1 629
	1 501		1 569	1 578	1 573				1 584
1 473 1 418	1 447	1 430 1 418	1 438	1 450 1 435	1 439	1 439	1 448		1 460 1 447
1 381 1 335	1 330	1 383 1 331	1 379 1 353 1 346 1 322	1 391 1 369 1 346 1 321 1 310	1 375	1 386 1 358 1 328	1 349	1 365	1 390 1 366
1 299 1 269 1 240 1 205	1 297 1 259 1 205	1 288 1 232	1 288 1 267 1 226	1 279 1 240 1 229	1 245	1 279 1 237 1 228 1 204	1 271 1 233	1 261 1 253	1 269 1 260 1 247 1 224
1 175	1 177	1 191 1 125	1 199 1 101	1 198 1 150 1 126		1 162	1 186 1 123	1 187	1 167 1 143 1 127 1 109
1 053	1 080 1 049	1 068 1 060	1 075 1 054 1 017	1 088 1 028 1 011	1 096 1 053 1 023		1 093	1 080 1 056	1 083 1 059 1 014
	965			937	987	948		965	963
	879	869		852		871	871		878
	752 704	724		795 762					
		697							
							597		

41	42	43	44	45	46	47	48	49
MLGA	DE	DES	HEX	DEdiAc	DESdiP	Z	Cort	HCort
1 738 1 716				1 757	1 763 1 754			1 714
1 666 1 625	1 619 1 608	1 609	1 613			1 644 1 615	1 694 1 648 1 618	1 644 1 610
1 579	1 591 1 513	1 590 1 514	1 598 1 516	1 503	1 504	1 587		
1 444 1 416	1 426	1 462 1 427	1 458 1 440		1 459	1 464	1 447 1 413	1 432
1 389 1 372 1 318	1 333	1 337		1 368	1 363	1 381 1 353 1 311	1 391	
1 260 1 245 1 231 1 205	1 247 1 205	1 282 1 247 1 204	1 218	1 218	1 210	1 259 1 200	1 269	1 271 1 237
1 123	1 171 1 102	1 173 1 114	1 174 1 107	1 196 1 164	1 157	1 167	1 186 1 159	1 133 1 115
1 037		1 012		1 019	1 076 1 019	1 096 1 075	1 075 1 064 1 037	1 047 1 006
972 953 930				910		989		942 900
881	853 834 826	851 831 805	847 830 804	865	895	840	875	865
	775	721	716					
627 612	649 620	646	646	679				
551	520	586	573 510					

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-D9(11)	Methyltestosterone-D9(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	Medroxprogesterone
39. MPA	Medroxyprogesterone acetate
40. MGA	Megestrol acetate
41. MLGA	Melengestrol
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