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QUALITY CRITERIA FOR THE DETECTION OF ANALYTES IN TEST SAMPLES WITH SPECIAL REFERENCE TO ANABOLIC AGENTS AND RELATED COMPOUNDS

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SUMMARY

In order to create a system for qualitative analysis, in which well defined limits of ambiguity can be set, criteria have been formulated for application to the identification method(s) used. Criteria are presented for separation techniques (thin-layer chromatography, gas chromatography, high-performance liquid chromatography) and for spectrometric methods (ultraviolet–visible spectroscopy via diode array, mass spectrometry, infrared spectroscopy), as well as general considerations for the whole procedure.

INTRODUCTION

The quality of quantitative analysis can be expressed in terms of accuracy: trueness and precision [1,2]. 'Trueness' refers to the closeness of agreement between the average value obtained from a large series of observations or test results and an accepted reference value. It is attributed to the bias of the procedure. 'Precision' refers to the closeness of agreement between independent observed values or test results obtained under prescribed conditions. It is attributed to the unavoidable random errors inherent in every test procedure. Two measures of precision, termed repeatability and reproducibility, are used for the minimum and the maximum variability in results, respectively.

For qualitative analysis these characteristics as such are irrelevant. Yet there is a need for unambiguous results in qualitative analysis as well, in particular in forensic control or in court. Therefore, to decide whether or not the presence of

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an analyte has been proved by a qualitative method, we have introduced 'quality criteria', aimed at preventing false-positive results [3]. 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 at issue. When consensus between experts is achieved about criteria, the conclusion holds, irrespective of the details of the analytical procedure leading to the analytical result. The criteria, presented here, are adopted for detecting residues of substances having hormonal or thyrostatic action in EEC control programs [4].

CRITERIA FOR THIN-LAYER CHROMATOGRAPHY (TLC)

The R_F value(s) of the analyte is (are) equal to the R_F value(s) of the standard material $\pm 3\%$ under the same conditions.

The visual appearance of the analyte is indistinguishable from that of the standard material. The centre of the nearest spot is separated from the spot due to the analyte by at least half the sum of the spot diameters.

For identification, additional co-chromatography in the TLC step is mandatory, causing spot intensification only. A new spot does not appear, and the visual appearance does not change.

For confirmation, two-dimensional TLC is mandatory.

CRITERIA FOR GAS CHROMATOGRAPHY (GC) AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The analyte elutes at the retention time of the standard material, under the same conditions.

The nearest peak maximum in the chromatogram is separated from the analyte peak by at least one full width at half maximum height.

For identification, additional co-chromatography in the chromatography step is mandatory, causing peak intensification only and the width at half maximum height is within $\pm 10\%$ of the original width. This requirement may be taken as fulfilled when the retention times are identical within 10% of the peak width at half maximum height.

CRITERIA FOR HPLC WITH UV-VIS SPECTRUM (DIODE ARRAY) DETECTION

The maximum absorption wavelength in the spectrum of the analyte is the same as that of the standard material ± 2 nm.

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

For identification, co-chromatography in the HPLC step is mandatory, causing peak intensification only.

CRITERIA FOR GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Criteria for GC

An internal standard is used, if a material suitable for this purpose is available. The ratio of the retention time of the analyte to that of the internal standard is the same as that of the standard analyte, within a margin of $\pm 0.5\%$. If this requirement is not fulfilled, or if no internal standard is used, co-chromatography is mandatory, in the case of which the retention time of the added analyte and the analyte already present in the sample coincide.

Criteria for low-resolution (LR) MS

All ions monitored derived from the analyte appear at a single retention time.

The intensities of at least four diagnostic ions are measured.

If the compound does not yield four diagnostic ions with the method used, identification of the analyte is based on the results of at least two independent GC–LRMS methods with different derivatization and/or ionization techniques, each producing two or three diagnostic ions.

The relative abundances of all diagnostic ions monitored from the analyte match those of the standard analyte.

The relative intensities of the ions detected, expressed as a percentage of the intensity of the ion of the base peak, are the same as those for the standard material, within a margin of $\pm 10\%$ (electron impact ionization mode) or $\pm 20\%$ (chemical ionization mode).

The molecular ion of the analyte in the multiple-ion detection spectrum is obtained, if possible at all.

Criteria for high-resolution MS; fragmentography

The accuracy of mass determination is $\geqslant 3$ parts per million (ppm).

The relative abundances of three or more diagnostic ions are the same as for the standard analyte, within a margin of $\pm 10\%$.

Criteria for high-resolution MS; accurate mass plus low-resolution natural isotope

The accuracy of mass determination is $\geqslant 3$ ppm.

The m/z value of the diagnostic ion is equal to the theoretical value of the standard analyte. If measurement of a single diagnostic ion is not specific, the natural isotope abundance ratio of the diagnostic ion is measured and has to be equal to the theoretical value within a specified margin (typically 5–15%). If in this way unambiguous identification cannot be derived, an additional diagnostic ion is measured.

CRITERIA FOR INFRARED (IR) SPECTROSCOPY

The definition of 'adequate peaks' is the absorption maxima in the IR spectrum of a standard material in the range 1800–500 cm^{-1}, having a relative absorbance not less than 12.5%, respectively 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, respectively to their peak baseline.

The definition of 'score' is the percentage of the adequate peaks of the standard material found in the IR spectrum of the analyte sample, within a margin of ± 1 cm^{-1}.

A minimum of six adequate peaks is required.

Absorption is present in all regions of the analyte spectrum that correspond to an adequate peak in the reference spectrum of the standard material.

The score is at least 50%. The presence of the remaining adequate peaks in the sample spectrum is presumed not to be excluded.

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

GENERAL CRITERIA FOR THE WHOLE PROCEDURE

The method is proved to be able to distinguish between the analyte and all known interfering materials in the appropriate matrix. The physical and chemical behaviour of the analyte during the analysis is indistinguishable from that of the corresponding standard material in the appropriate matrix.

GENERAL CRITERIA FOR SEPARATION TECHNIQUES

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

If no appropriate reference samples are available, quality control samples having a known content of added standard analyte close to that of the expected analyte content of the samples are subjected to the same derivatization procedure as the samples, including any post-derivatization clean-up.

CRITERION FOR OFF-LINE PHYSICAL AND/OR CHEMICAL PRECONCENTRATION PURIFICATION AND SEPARATION, IF APPLIED

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

CRITERION FOR ON-LINE CHROMATOGRAPHIC SEPARATION, IF APPLIED

The analyte elutes at the retention time that is characteristic for the standard material in the appropriate matrix material.

CONCLUSION

The criteria, as stated here, are a helpful tool to achieve objective and unambiguous conclusions about the presence or absence of an analyte, and to avoid false-positive results. They do not present, however, a measure for the value of a

method of analysis, which is another important parameter of qualitative analyses; this parameter can be expressed in terms of an 'uncertainty factor', as will be reported elsewhere [5].

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