CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Discussion Paper

(Prepared by Iran)

Background

The forty-ninth session of Codex Committee on Pesticide Residues (CCPR49) China 24-29 April 2017, proposed that the delegations of Iran would prepare a discussion paper on revise Codex GL56 **"GUIDELINES ON THE USE OF MASS SPECTROMETRY (MS) FOR IDENTIFICATION, CONFIRMATION AND QUANTITATIVE DETERMINATION OF RESIDUES".**

Introduction

Guidelines on the use of mass spectrometry (MS) for identification, confirmation and quantitative determination of residues focusing on:

1. Layout and sequence of clause due to Codex Standard frame.
2. More focusing on facts about MS spectrometry as a powerful confirmation and quantitation method for determination pesticide residues especially in multi residue method.

Discussion and Conclusions

The purpose of this discussion paper is to propose options and reasons of revising codex GL 56 by explaining confirmatory tests, focusing on mass spectroscopy.

The core of the document is divided in three sections:

1- General principles of confirmatory tests in determination of pesticide residues especially in multi residue methods and demonstrating advances of MS technique among other confirming techniques both in GC applicable and HPLC applicable pesticides.

2- Criteria for selection of recognition ions for identification, confirmation and quantitative detection

3- Interpretation of results and Identification and Confirmation of residues.

4- Advances and limitation of quantification of identified residues.

The proposed layout of the revised guidelines 56 has been attached to this discussion paper. To provide this proposed draft with regard to GL 56 it is necessary to appreciate the work ad that earlier group had done to the write the said guideline we have used following reference :

References

1- CAC/GL 56-2005, use of mass spectrometry (MS) for identification, confirmation and quantitative determination of residues.

2- Soboleva E. Ambrus A., Application of system suitability test for quality assurance and performance optimization of a gas chromatographic system for pesticide residue analysis, J. Chromatogr. A. 1027. 2004. 55-65.

3- Lantos J., Kadenczki L., Zakar F., Ambrus A. Validation of gas chromatographic Databases for qualitative identification of active ingredients of pesticide residues in Fajgelj A. Ambrus A. (eds) Principles of Method Validation, Royal Society of Chemistry, Cambridge, 2000, pp 128-137.

4- SANTE/11813/2017, Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed

5- Boris L. Milman, Identification of chemical compounds, Trends in Analytical Chemistry, Vol. 24, No. 6, 2005

6- FrançoisAndréKatia K.GDe WaschHubert FDe Brabander, Trends in the identification of organic residues and contaminants: EC regulations under revision, TrAC Trends in Analytical Chemistry, Volume 20, Issue 8, August 2001, Pages 435-445

7- Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C(2002) 3044)

8- William C. Brumley and James A. Sphon, Regulatory Mass Spectrometry

GUIDELINES ON THE USE OF MASS SPECTROMETRY (MS) FOR IDENTIFICATION, CONFIRMATION AND QUANTITATIVE DETERMINATION OF RESIDUES

CAC/GL 56-2017(1st revision)

Introduction

When analyses are performed for monitoring or enforcement purposes, it is particularly important that confirmatory data are generated before reporting on samples containing residues of pesticides that are not normally associated with that commodity, or where MRLs appear to have been exceeded. Samples may contain interfering chemicals that may be misidentified as pesticides.

It can be argued that quantification of analyte is meaningless without confirmation of its identity, while in some cases, like that of banned compounds or qualitative analysis, confirmation is only needed or it is more important than quantification.

Confirmatory tests may be quantitative and/or qualitative but, in most cases, both types of information will be required. Particular problems occur when residues must be confirmed at or about the limit of determination, although it is difficult to quantify residues at this level, it is essential to provide adequate confirmation of both level and identity.

The need for confirmatory tests may depend upon the type of sample or its known history. In some crops or commodities, certain residues are frequently found. For a series of samples of similar origin, which contain residues of the same pesticide, it may be sufficient to confirm the identity of residues in a small proportion of the samples selected randomly. Similarly, when it is known that a particular pesticide has been applied to the sample material, there may be little need for confirmation of identity, although a number of randomly selected results should be confirmed. Where “blank” samples are available, these shall be used to check the occurrence of possible interfering substances.

Conventionally, for the confirmation of positive results for pesticide residues in food or any environmental compartment different approaches have been adopted, such as gas chromatography with two different detectors or two columns of different polarities, combination of two chromatographic techniques or chemical reaction followed by the analysis of the derivative. Other means of confirmation, such as characteristic chromatographic pattern, might be alternatively applied. For example, four isomers of cypermethrin form a specific pattern, which, combined with retention times can serve as additional evidence of cypermethrin identity. In similar cases, however, care should be taken when reisomerisation is possible[1].

However, these classical confirmatory approaches do not provide sufficient structural information about the analyte.

Confirmatory methods should provide as much as possible structural information about the analyte, which is only possible by applying spectrometric techniques (e.g. MS, IR). Therefore, most of the documents setting the confirmation criteria for residues and contaminants describe the combination of a chromatographic technique with mass spectrometry as the main confirmatory tool.

Scope

This guideline deals with general principle of application of mass spectrometer (MS) in Identification, confirmation and quantitative determination of pesticide residues and should be read in conjunction with all relevant method of analysis for pesticide residues.

General principles

Analysis of pesticide residues with multi-residue methods generally consists of two phases: screening and confirmation. The process is schematically depicted in Fig. 1. The first phase comprises establishment of those pesticide residues that are likely to be present from interpreting the raw data, avoiding false negatives as much as possible. The second phase is the confirmation, which focuses on the pesticides found in phase1. The use of the results to be reported, and consequent management decision determines the efforts put in the confirmatory process. The choice of the technique used for confirmation depends on their availability, time and cost. They are based on either further interpretation of chromatographic and mass spectrometric data, alternative methods using different physico-chemical properties of the compound, or a combination of various separation and detection methods. Some alternative procedures for confirmation are given in Table 1.

[1] EN12393-3-2013: Foods of plant origin – multiresidue methods for the determination of pesticide residue by GC or LC/MS. Part 3: Determination and confirmatory tests

Selection of recognition ions for identification, confirmation and quantitative detection

Mass-spectrometric detection shall be carried out by employing MS-techniques using full mass spectra (full scans) or selected ion monitoring (SIM) or Selected Reaction Monitoring (SRM), or other suitable MS or MS-MS^n techniques in combination with appropriate ionization modes. In case of high-resolution mass spectrometry (HRMS), the resolution shall typically be greater than 10000 for the entire mass range at 10 % valley.

Reference spectra for the analyte should be generated using the same instruments and techniques employed for analysis of the samples. If major differences are evident between a published spectrum and that generated within the laboratory, the latter must be shown to be valid.

When full scan spectra are recorded in single mass spectrometry, a minimum of four ions shall be present with a relative intensity of ≥ 10 % of the base peak. The molecular ion shall be included if it is present in the reference spectrum with a relative intensity of ≥ 10 %. Computer-aided library searching[2] may be used. In this case, the comparison of mass spectral data in the test samples to that of the calibration solution has to exceed a critical match factor. This factor shall be determined during the validation process for every analyte on the basis of spectra for which the criteria described below are fulfilled. Variability in the spectra caused by the sample matrix and the detector performance shall be checked.

In case of full scan measurement, careful subtraction of background spectra by deconvolution or other algorithms, may be required to ensure that the resultant spectrum from the chromatographic peak is representative. Whenever background correction is used, this must be applied uniformly throughout the batch and should be clearly recorded.

If mass spectrometric determination is performed by SIM, 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.

Many facts have to be considered when selecting the characteristic ions for SIM method development. Notorious interferences, such as ions known to be abundant in the environment, like phthalates (m/z 149), column artifacts (m/z 73, 207, 221, 281, 327), matrix, background, loss of specific moiety (m/z 18) etc. should not be included when method for SIM is developed.

Identification and Confirmation of results

Extracted ion chromatograms of sample extracts should have peaks of similar retention time, peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch. Chromatographic peaks from different selective ions for the analyte must fully overlap. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon for identification.

One of the problems in pesticide residue analysis is the lack of a sufficient number of ions with the required abundances in the mass spectra of some pesticides. For example, electron impact ionization mass spectra of bitertanol, methoxychlor, phosmet yields quantification ions only with abundance about or lower than 10% of the base peak, which cannot be used for quantification purposes due to high uncertainty of measurement. Besides, they will significantly increase LOQ as it will be discussed below. In some other cases, such as dimethoate, mevinphos and fenthion diagnostic ions are not specific and ion traces of identification masses often overlap with matrix components. For example, three ions with m/z 109, 127, 192 can be selected for identification of mevinphos in SIM mode, but two of them (109, and 127) often appear in the overlapping co-extracts[3].

Different types and modes of mass spectrometric detectors provide different degrees of selectivity and specificity, which relates to the confidence in identification. The requirements for identification are given in Table 2. They should be regarded as guidance criteria for identification, not as absolute criteria to prove the presence or absence of an analyte.

[2] The Automated Mass Spectral Deconvolution and Identification System (AMDIS) is a computer program that extracts spectra for individual components in a GC/MS data file and identifies target compounds by matching these spectra against a reference library.

[3] Soboleva E. Ahad K. Ambrus A. Applicability of some MS criteria for the confirmation of pesticide residues, Analyst, 129, 1123-1129, 2004.

Quantification

When using selected ion monitoring (SIM), tolerance intervals of ion ratios and retention times based on injection of pesticide standard in pure solvent at the concentration close to the critical level should have been established at this point. 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 comparable concentrations and measured under the same conditions, within the tolerances ±30%. When two (or three) selected ion ratios are within the established tolerance intervals the residue is confirmed.

For a small number of pesticides the mass spectrum may only exhibit one specific ion. In this case alternative confirmation should be sought.

When the ions detected still indicate the possible presence of a residue, the result may be reported as "tentatively identified". However, when the result would lead to regulatory action, or results would be used for other purposes (e.g. dietary intake assessment) further confirmation of analyte identity shall be sought. This can be achieved with the same instrumentation, by injecting matrix-matched standards of the suspected analyte, in order to compensate for matrix influence on ion ratios. In this case, subsequent injections of matrix matched standard and suspected sample has to be made. The deviation of RRT of analyte in standard and suspected peak in sample should typically be less than 0.1 %. Two ion ratios measured in a sample should be within the tolerance interval calculated based on the ion ratios in matrix matched standard. The residue is considered to be confirmed if it complies with the general rule stated above. If the ion ratios are not within the tolerance intervals, additional confirmation of identity may be obtained by the use of alternative analytical techniques. Examples are listed in Table 1.

Confirmation of residues detected following separation by HPLC is generally more problematic than where gas chromatography is used. LC-MS can provide good supporting evidence but, because the spectra generated are generally very simple, showing little characteristic fragmentation, results produced from LC-MS are unlikely to be definitive. LCMS/MS is a more powerful technique, combining selectivity with specificity, and often provides good evidence of identity. LC-MS techniques tend to be subject to matrix effects, especially suppression, and therefore confirmation of quantity may require the use of standard addition or isotopically-labelled standards. Derivatisation may also be used for confirmation of residues detected by HPLC (Table 1).

Further confirmation by mass spectrometry can be accomplished by acquisition of the complete electron impact mass spectrum (in practice generally from m/z 50 to beyond the molecular ion region). The absence of interfering ions is an important consideration in confirming identity. Additional confirmation of identity may be obtained by (i) the use of an alternative chromatographic column; (ii) by the use of an alternative ionization technique (e.g. chemical ionization); (iii) by monitoring further reaction products of selected ions by tandem mass spectrometry (MS/MS or MS^n); or (iv) by monitoring selected ions at increased mass resolution.

Whenever chromatographic techniques are used in screening or confirmation, proper settings of the retention time windows is pivotal. Care should be taken that the instrument is adjusted correctly before starting the analysis; a system suitability test should be performed prior to each batch of analysis[4]. Retention times data base should be adjusted for the current conditions[5]. In phase 1, tolerance intervals of 1.5 to 3% of the absolute retention time may be applied for capillary GC depending on the peak shape. For confirmation of the retention time, the absolute tolerance intervals will increase at higher retention time. The tolerance interval should be less than 1 sec for an RT less than 500 sec. For retention times between 500 and 5000 sec. an interval of 0.2% RRT is recommended. For higher retention times 6 sec. is a suitable interval.

[4] Soboleva E. Ambrus A., Application of system suitability test for quality assurance and performance optimization of a gas chromatographic system for pesticide residue analysis, J. Chromatogr. A. 1027. 2004. 55-65.

[5] Lantos J., Kadenczki L., Zakar F., Ambrus A. validation of gas chromatographic Dtabases for qualitative identification of active ingredients of pesticide residues in Fajgelj A. Ambrus A. (eds) Principles of Method Validation, Royal Society of Chemistry, Cambridge, 2000, pp 128-137.

Table 1. Detection methods suitable for screening and confirmation of residues.

		Phase 1-Screening							
		GC with capillary column – ECD, NPD, FPD, PFPD	GC-MS	LC-MS	LC-DAD or scanning UV	LC-UV/VIS (Single wavelength)	LC- fluorescence	GC with packed column – ECD, NPD, FPD	TLC- enzyme, fungal growth or chloroplast inhibition
Phase2- Confirmation	GC-capillary column – ECD, NPD, FPD, PFPD	x[1]	x[1]	x	x	x	x	x	x
	GC-MS	x	x[1,2]	x	x	x	x	x	x
	LC-MS	x	x		x	x	x	x	x
	Full scan techniques	x	x	x	x	x	x	x	x
	$(MS)^n$, HRMS, alternative ionization techniques	x	x	x	x	x	x	x	x
	LC-DAD or scanning UV	x	x	x		x	x	x	x
	LC-UV/VIS (single wavelength)	x	x				x	x	x
	LC- fluorescence	x	x		x	x		x	x
	TLC – enzyme, fungal growth or chloroplast	x	x	x	x	x	x	x	x[2, 3]
	Derivatisation	x	x	x	x	x	x	x	x
	Specific isomers profile	x	x	x	x	x	x	x	

1 - Either the column of different polarity, which results in different elution order of the residues and contaminants eluting in the vicinity to the peak of interest, or another specific detector shell be used.

2- The same GC-MS technique can be used for the confirmation if different ions are selected or tolerance intervals are established based on matrix matched solutions.

3 - Mobile or stationary phase of different polarity shall be used.

Table 2. Identification requirements for different MS techniques

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	Other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[e)] Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within ±30% (relative) of average of calibration standards from same sequence
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 productions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a,b, c]	
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm[a,c] *plus* 1 MS/MS production[d]	

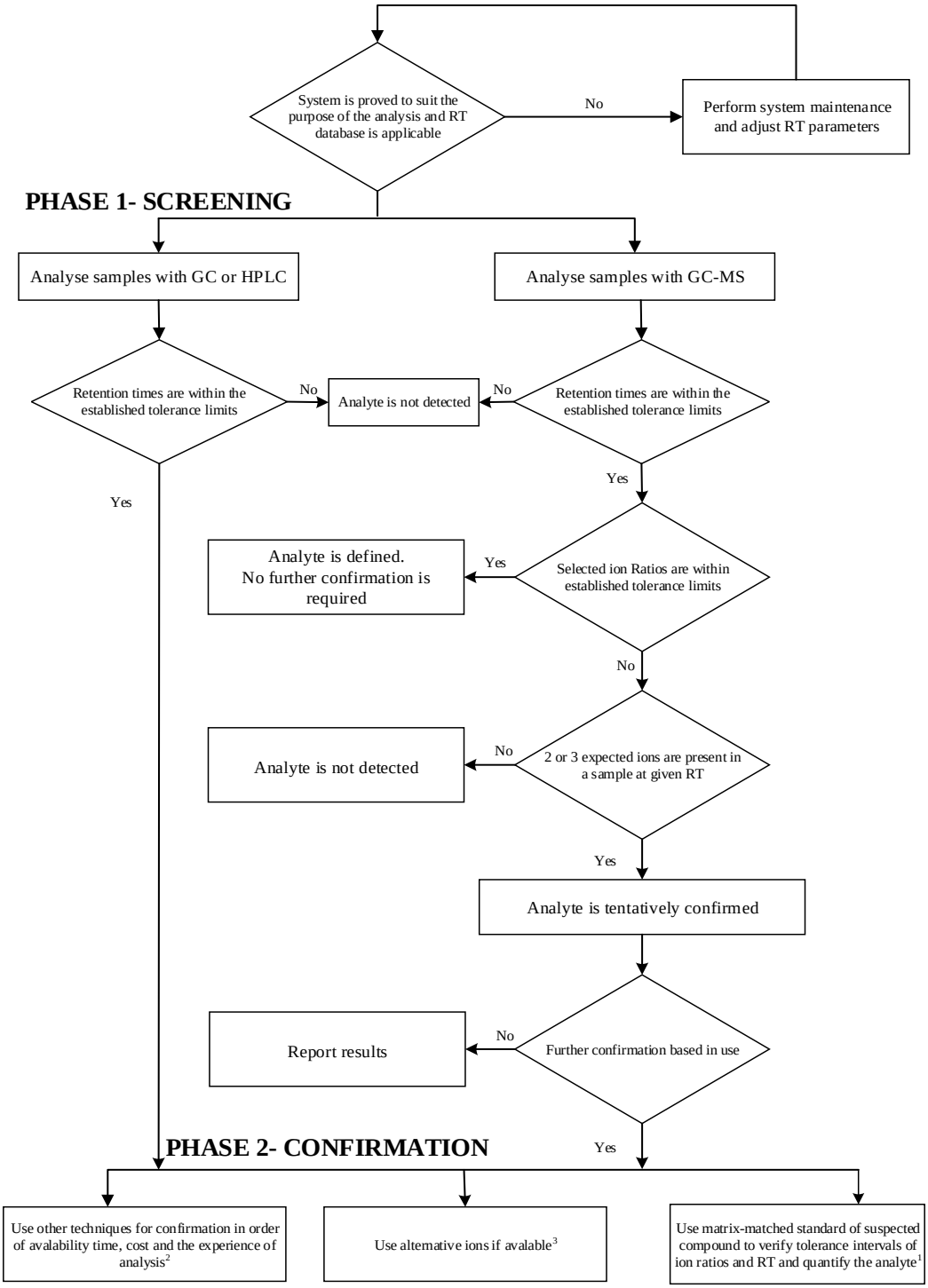
[a)] Preferably including the molecular ion, (de)protonated molecule or adduct ion

[b)] Including at least one fragment ion

[c)] < 1 mDa for m/z < 200

[d)] No specific requirement for mass accuracy

[e)] In case noise is absent, a signal should be present in at least 5 subsequent scans



1- Unusual values including banned substances, MRL violation or study requirements as in e.g. exposure assessment

2- Refer to table 6 for other means of confirmation

3- For a small number of pesticides the mass spectrum may only exhibit one specific ion. In this case alternative confirmation should be sought.

Figure 1. Schematic Representation of Screening and Confirmation (Phase 1 and Phase 2) for Pesticide Residues

GLOSSARY OF TERMS

Confirmation	The process of generating sufficient evidence to ensure that a result for a specific sample is valid. Analytes must be identified correctly in order to be quantified. The identity and quantity of residues should be confirmed. It is impossible to confirm the complete absence of residues. Adoption of a "reporting limit" at the LCL avoids the unjustifiably high cost of confirming the presence, or absence, of residues at unnecessarily low levels.
MS/MS	Tandem mass spectrometry, here taken to include MS^n. An MS procedure in which ions of a selected mass to charge ratio (m/z) from the primary ionization process are isolated, fragmented usually by collision, and the product ions separated (MS/MS or MS^2). In ion-trap mass spectrometers, the procedure may be carried out repetitively on a sequence of product ions (MS^n), although this is not usually practical with low-level residues.
Validation	The confirmation by examination and the provision of effective evidence that the particular requirements of a specific intended use are fulfilled.
Determination	A quantitative result from a method that meets the acceptable performance criteria for the quantitative purpose of the analysis (e.g., chromatography with an element-selective detector).
Identification	A qualitative result from a method capable of providing structural information (e.g., using mass spectrometric (MS) detection) that meets acceptable criteria for the purpose of the analysis.
Full scan	When mass spectrometric determination is performed by the recording of full scan spectra, the presence of all measured diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and isotope ions) with a relative intensity of more than 10 % in the reference spectrum of the calibration standard is obligatory.
Selected ion monitoring (SIM)	When mass spectrometric determination is performed by fragmentography, the molecular ion shall preferably be one of the selected diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and all their isotope 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 shall be ≥ 3:1.
Selected reaction monitoring (SRM)	Data acquired from one or more specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry. Note 1: Selected reaction monitoring in multiple-stage mass spectrometry is known as consecutive reaction monitoring. Note 2: Selected reaction monitoring applied to multiple product ions from one or more precursor ions is known as multiple reaction monitoring.
Multiple Reaction Monitoring (MRM)	Application of selected reaction monitoring to multiple product ions from one or more precursor ions. Note: This term should not be confused with consecutive reaction monitoring, which involves the serial application of three or more stages of selected reaction monitoring.