

METHOD VALIDATION AND QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

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Pesticides: Method Validation and Quality Control

- The guidance in 10684/2009 is intended for laboratories control or in the monitoring of pesticide residues in food involved in official and feed in the European Union.
- The document describes the method validation and analytical quality control (AQC) requirements
 - to support the validity of data used for checking compliance with maximum residue limits (MRLs),
 - enforcement actions,
 - or assessment of consumer exposure to pesticides

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The key objectives of SANCO 10684/2009 are:

- to provide a harmonized cost-effective quality assurance system in the EU
 - to ensure the quality and comparability of analytical results
 - to ensure that acceptable accuracy is achieved
 - to ensure that false positives or false negatives are not reported
 - to support compliance with ISO/IEC 17025
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- This document is complementary and integral to the requirements in ISO/IEC 17025
 - Accreditation bodies shall accept that laboratories work according to the rules of SANCO 10684/2009

Advisory Board for the SANCO Document



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Main contents of SANCO 10684/2009 are:

- Sampling, transport, processing and storage of samples (6-9)
- Sample preparation and processing prior to analysis (10-14)
- Identity, purity, and storage of standards (15)
- Preparation and storage of stock standards (16-19)
- Preparation, use and storage of working standards (20-21)

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- Testing and replacement of standards (22-23)
- Extraction conditions and efficiency (24)
- Extract concentration and dilution to volume (25-27)
- Avoiding contamination (28-32)
- Minimising Interference (33-34)

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Analytical calibration, representative analytes, matrix effects and chromatographic integration:

- General requirements (35-37)
- Calibration (38-41)
 - Residues below the LCL (LCL corresponding to RL) reported as <RL
 - Difference between two levels is less than a factor of 4
 - With 3 or more levels an appropriate calibration function may be calculated and used between lowest and highest calibrated levels

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Representative analytes (42-43)

- Each determination system should be calibrated with all the targeted analytes for every batch of analyses
- If this requires a disproportionately large number of calibrations, the determination system must be calibrated with a minimum number of representative analytes
- representative analytes must be chosen very carefully according to the probability of finding residues in the sample and the physico-chemical characteristics
- Number of representative analytes = 15 plus 25% of the total number of analytes for each determination system.
 - Example: 60 Analytes => 15 + 15 (25 % of 60) = 30

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Representative analytes (42-43)

Table 1. Minimum frequencies for calibration

	Representative analytes	All other analytes
Minimum frequency of calibration	In each batch of analyses. At least one calibration point corresponding to the reporting limit.	Within a rolling programme at least every third month* At least one calibration point corresponding to the reporting limit See also paragraph 43.

Where an analyte that is not a representative analyte is detected in a sample, the result must be considered tentative until calibrated

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Matrix effects and matrix-matched calibration (44-46)

- potential for matrix effects to occur should be assessed at method validation.
- hints for minimising
- use of analyte protectants

Description of using standard addition (47-48)

- 3 or more test portions
- amount of analyte added should be between one and five times the estimated amount of the analyte in the sample
 - Matrix effects and recovery will be corrected
 - Adding analytes to extracts corrects for matrix effects, only
 - No real standard addition

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Effects of pesticide mixtures on calibration (49)

Calibration for pesticides that are mixtures of isomers (50)

Calibration using derivatives or degradation products (51)

Chromatographic integration (52-53)

- Integration must be checked by the analyst
- calibration by mixed isomer (or similar) standards may utilise summed peak areas, summed peak heights, or measurement of a single component, whichever is the more accurate

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Analytical method validation and performance criteria (54-58)

Qualitative screening methods (54)

- Makes sense for analytes potentially have low probability to be present in the samples
- Validation in case of qualitative screening methods is focused on detectability.
- The detection is the lowest spiking level for which has been demonstrated that a certain analyte can be detected (not necessarily identified) in at least 95% of the samples (i.e. a false-negative rate of 5% is accepted) at the concentration of interest
- When strictly used as qualitative method, there are no requirements with regard to linearity and recovery.

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Initial method validation (55-58)

- Within-laboratory method validation should be performed to provide evidence that a method is fit for the purpose
- Method validation must be supported and extended by method performance verification during routine analysis (analytical quality control and on-going method validation).
- All procedures (steps) that are undertaken in a method should be validated, if practicable.
- representative matrices may be used.
 - As a minimum, one representative commodity from each commodity group as described in Annex I must be validated
 - When the method applied in routine for a wider variety of matrices, complementary, on-going QC- and validation data should be acquired during the routine analyses.

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Representative Matrices (Annex I)

Products of animal origin

Commodity groups	Commodity categories	Typical representative commodities included in the category
Meat	Red meat White meat Fish Offal *) fat from meat	Beef, pork, lamb, game, horse Chicken, duck, turkey Cod, haddock, salmon, trout, Liver, kidney
Milk and milk products	Milk Cheese Yogurt Cream Butter	Cow, goat and buffalo milk Cow, goat cheese
Eggs	Eggs	Chicken, duck, quail, goose eggs
Honey	Honey	

*) Offal (liver, kidney) should be validated separately, if necessary

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Initial method validation (55-58)

- A practical approach to the validation procedure is presented in [Appendix A](#).
- The method must be tested to assess for
 - sensitivity,
 - mean recovery (as a measure of trueness or bias),
 - precision, and
 - limit of quantification (LOQ).
- A minimum of 5 replicates is required (to check the precision) at both
 - the reporting limit (to check the sensitivity of the method), and
 - at least another higher level, perhaps an action level, for example the MRL

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Initial method validation (55-58)

- **mean recoveries for each representative commodity must be in the range 70-120%, with a RSD_r ≤ 20%**
- Other approaches to demonstrate that the analytical method complies with performance criteria may be used, provided that they achieve the same level and quality of information.
- Where the residue definition incorporates two or more analytes, if possible, the method should be validated for all analytes included in the residue definition.

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Acceptability of analytical method performance—extended method validation (59)

- for all compounds to be sought using the method
 - Recovery within the range 70–120%,
 - repeatability $RSD_r \leq 20\%$
 - within laboratory reproducibility $RSD_{WR} \leq 20\%$
- Exceptionally, where recovery is low but consistent, a mean recovery below 70% may be acceptable.
 - However, a more accurate method should be used, if practicable.

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On-going performance verification (routine recovery determination) (60-63)

Table 2. Frequency for routine recovery (performance verification)

	Representative analytes	All other analytes
Minimum frequency of recovery	10% of representative analytes (at least 5 per detection system) in each batch of analyses	Within a rolling programme to include all other analytes at least every 12 months, but preferably every 6 months
	Within a rolling program covering all representative analytes as well as different types of commodities, at least at the level corresponding to the reporting limit.	At least at the level corresponding to the reporting limit.

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Acceptability of analytical performance for routine recoveries (65-66)

- The mean recovery is calculated from one commodity group.
- Acceptable limits for a single recovery result should normally be in the range of the mean recovery $\pm 2 \times \text{RSD}$
 - may be adjusted using within laboratory reproducibility (routine on going recovery) data or repeatability (initial validation)
- A range of 60-140 % is acceptable in routine multi residue analysis.
- If a significant trend occurs in recovery, or potentially unacceptable (RSD outside $\pm 20 \%$) results are obtained, the cause(s) must be investigated.

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Acceptability of analytical performance for routine recoveries (65-66)

- Data on **numerical exceedences of the MRL** residues must be supported by individual recovery results in the same batch
 - within the range of the mean recovery (70-120 %) $\pm 2 \times \text{RSD}$, at least for the confirmatory analyses.
 - If recovery within this range cannot be achieved, enforcement action is not necessarily pre-cluded, but the risk of relatively poor accuracy must be taken into account.
 - **It is recommended to correct for recovery preferably by using standard addition or isotopically labelled standards in all cases of violation**

Proficiency testing and analysis of reference materials (67-68)

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Confirmation of results (69-80)

- Positive results usually require additional confirmation
- Suspected MRL exceedances or unusual residues must be identified.
- Identification with Non-MS-Detektors
- Requirements for chromatography
- Requirements for mass spectrometry (MS)
 - Reference spectra should be generated using the instruments and techniques employed for analysis of the samples
 - The (quasi) molecular ion is a diagnostic ion that should be included in the measurement and identification procedure
 - The ion with the best signal-to-noise ratio and no evidence of significant chromatographic interference should be used for quantification.

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Table 3 Identification requirements for different types of mass spectrometers

MS mode:	Single MS (standard mass resolution)	Single MS (high resolution/high mass accuracy)	MS/MS
Typical systems (examples)	quadrupole, ion trap, time-of-flight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole ion trap, hybride MS (e.g. Q-TOF, Q-trap)
Acquisition:	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for identification:	≥ 3 diagnostic ions, (preferably including quasi molecular ion)	≥ 2 diagnostic ions (preferably including the quasi molecular ion). Mass accuracy < 5 ppm. At least one fragment ion.	≥ 2 product ions
Ion ratio(s):	according to Table 4		

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Table 4. Default recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques².

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MSn, LC-MS, LC-MSn (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10%	± 50 %	± 50 %

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Reporting of results

Expression of results (81)

Calculation of results (82-84)

- Residues data do not have to be adjusted for recovery, when the mean recovery is in the range of 70-120%.
- If residues data are adjusted for recovery, then this must be stated.

Rounding of data (85)

Qualifying results with uncertainty data (86-89)

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Interpretation of results **for enforcement purposes** (90-94)

- Default expanded uncertainty figure of 50%, in general covers the inter-laboratory variability between the European laboratories
 - recommended to be used by regulatory authorities in cases of enforcement decisions (MRL-exceedences).
- Laboratory proves its own calculated expanded uncertainty to be less than 50%.

