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REPORT OF THE IN-SESSION WORKING GROUP ON GUIDELINES FOR MONITORING THE STABILITY AND PURITY OF REFERENCE MATERIALS AND RELATED STOCK SOLUTIONS OF PESTICIDES DURING PROLONGED STORAGE

(Prepared by India, Chair of the Virtual Working Group)

SUMMARY OF THE IN-SESSION WORKING GROUP (ISWG)

1. An ISWG meeting for Agenda Item 7, Guidelines for monitoring the purity and stability of reference materials and related stock solutions of pesticides during prolonged storage was held on 9th September 2025. India as the Chair of the electronic working group (EWG) on Monitoring the stability and purity of multiclass pesticide reference materials and their stock solutions during prolonged storage, on behalf of the co-chairs Singapore, Canada and Iran (Islamic Republic of), briefly presented the item, the background, work done by the EWG and apprised the members about the changes made in the guidelines based on the comments received in reply to circular letter CL 2025/38-PR and the CRDs.

2. Appendix I of PR56/CRD03 Rev.1 with the changes incorporated shown as track change was displayed and members were invited to provide general and specific comments related to the guidelines or any section thereof. Based on the deliberations held during the ISWG and comments from Australia, Canada, Chile, Colombia, Germany, Ghana, Kenya, Singapore, USA, the following key modifications have been incorporated:

   a) As proposed by Colombia, one additional method has been included in Approach 3 in accordance with ISO 33405 for verification of stability of mixed pesticide standard solutions of reference materials (RMs) procured from reference material providers (RMPs).

   b) The Method 1 and 2 of Approach 3 for verification of stability of mixed pesticide standard solutions of RMs during prolonged storage is applicable to mixed pesticide standard solutions of RMs purchased from the RMPs as well as mixed pesticide standard solutions of RMs prepared by laboratories using individual RMs procured by the laboratories from the RMPs.

RECOMMENDATION

3. CCPR56 is invited to consider the proposed guidelines as set out in Appendix I and provide general and specific comments on the guidelines including its readiness for advancement to Step 8 for final adoption by CAC48 (November 2025).

APPENDIX I

GUIDELINES FOR MONITORING THE STABILITY AND PURITY OF REFERENCE MATERIALS AND RELATED STOCK SOLUTIONS OF PESTICIDES DURING PROLONGED STORAGE

PREFACE

1. Pesticide residues in agricultural crops and food commodities have become a worldwide ~~agricultural trade~~ food safety and trade concern, which has led to enforcement of strict pesticide regulations. More than 1200 pesticides are available globally to control the pests on different agricultural crops and food commodities. Analyses of pesticides at trace levels in the food chain require the use of specific Reference Materials (RMs) of known chemical purity manufactured by the Reference Material Producers (RMPs) to ensure the reliability of the test results. Accurate determination of pesticide residues in agricultural crops and food commodities is important for food safety control and fixation of Maximum Residue Limits (MRLs) of pesticides, thereby overcoming the related trade barriers. RMs with specified purity are also required for accurate qualitative and quantitative analysis of pesticide active ingredient(s) in technical products, formulations, and stock solutions.

2. Limited shelf life, diminishing purity, and high recurring cost of RMs act as major impediments to performing regular pesticide residue analysis. These problems are magnified for multi-pesticide residue analysis by testing laboratories in developing countries as they are required to allocate a large part of their funds for frequent procurement of expensive RMs. Furthermore, the use of RMs is restricted by the expiry dates specified by the RMPs in the reference material document (e.g., certificate of analysis (CoA) or product information sheet), which provides the value for purity, expiry date, and measurement uncertainty of the RMs as per ISO 33401. Many times, laboratories cannot afford the frequent purchase of high-cost RMs for their pesticide residue control work.

3. Moreover, due to supply chain constraints, some laboratories may receive RMs close to their expiry date, as mentioned in the reference material document. In such situations, the laboratories are forced to buy new standards and prepare new stock solutions more frequently than necessary. This leads to enormous amount of work and increased laboratory costs~~, especially for compounds for which stability is well understood~~. Additionally, shipping RMs by the suppliers to laboratories increases the acquisition time for procurement (a few weeks to months), creating hurdles in ~~sustainable~~ sustaining pesticide residue control programs.

4. There are many RMs that remain stable even after the expiry dates stated in the reference material document with no significant change in purity. Some studies[i-iii] ~~1-3~~ have also reported that if RMs are stored at better storage conditions than recommended by the manufacturer, provided that these conditions do not contradict those indicated by the RMP in the reference material document, the RMs are stable for much longer than the expiry dates indicated by the RMPs. Such RMs may technically be allowed to be used beyond their expiry dates if laboratory checks are in place to demonstrate that they are stable and continue meeting the purity requirements. However, the absence of guidance procedures for monitoring the stability and purity of RMs prevents their use beyond the expiry ~~dates~~ dates under the ISO/IEC 17025 laboratory quality system.

5. This document represents a crucial step towards developing comprehensive harmonized guidance enabling the laboratories to monitor the stability and purity of the pesticide RMs and their stock solutions during prolonged storage. The document aims to guide the laboratories in monitoring the stability and purity of RMs for their possible use beyond their expiry dates and for continued use of stock solutions that retain their stability and purity.

SCOPE AND OBJECTIVE

6. The purpose of this document is to furnish a framework that would assist the laboratories in monitoring the stability and purity of reference materials (RMs) of pesticides during prolonged storage and identifying expired RMs as indicated by the ~~CoAs~~ reference material document of RMPs but with ~~continued~~ demonstrated continuing stability and purity through robust analytical protocols so that such materials that retain their purity as per the reference material document even after expiry ~~can~~ may continue to be used as valid RMs. Another aspect of the proposed ~~work~~ framework is to monitor the stability of the stock solutions used for pesticide residue analysis so that those solutions that ~~continue~~ are proven to be valid ~~can~~ may be used for ~~the~~ accurate and reliable determination of pesticide residue levels.

7. This document applies to RMs of pesticide standards of known purity specified by a RMP, including individual RMs, stock solutions of individual RMs, and mixed pesticide standard solutions of RMs purchased from the RMPs or prepared by the laboratories from the individual RMs procured by the laboratories from the RMPs~~RMs purchased as mixtures~~.

8. These guidelines may~~will~~ enable the pesticide residue laboratories and pesticide quality control laboratories to overcome the constraints associated with short expiry periods of RMs as shown in the RMP documents and use them beyond their expiry dates ~~mentioned in the reference material document~~as indicated by RMPs. After the expiration dates of RMPs, the RMs retaining the purity specified in the reference material document ~~can~~ may be used as RMs or as quality control materials (QCM) for the analysis of pesticides, provided that these RMs are stored under desirable conditions ~~specified in the guidelines~~ (low temperature and dark conditions)~~according to the manufacturer's instructions~~. RMs that do not remain stable and do not show acceptable purity during prolonged storage shall not be used by laboratories for pesticide residue testing/quantitative purposes, as accurate results may not be obtained.

9. The guidelines cover the storage conditions that shall be maintained and quantitative measurements that shall be performed to monitor the stability and purity of RMs and their stock solutions before and beyond their expiration period.

GENERAL CRITERIA

10. The analysis shall be conducted in laboratories in compliance with the general criteria for testing laboratories laid down in ISO/IEC 17025[iv], with the scope relevant to the measurement concerned.

11. ~~The stability of mixtures of RMs may be evaluated under these guidelines only if the mixture is purchased from the RMP, who can certify the purity and stability of each of the individual components.~~The RMs shall be procured from an RMP accredited as per ISO 17034 to ensure analytical traceability or from a National Metrology Institute recognized by peers or designated by countries.

12. The stability of ~~mixtures of RMs~~mixed pesticide standard solutions that may be evaluated under these guidelines ~~only if~~ include the ~~standard~~ mixed pesticide standard solutions~~mixture~~ ~~is~~ purchased from the RMP as well as the mixed pesticide standard solutions~~standard mixture~~ prepared by pesticide residues laboratories by using RMs purchased from RMPs, who ~~can~~ may certify the purity and stability of each of the individual components.

~~13. The RMs shall be procured from an RMP accredited as per ISO 17034 to ensure analytical traceability or from a National Metrology Institute recognized by peers or designated by countries.~~

~~14.~~13. To ensure metrological traceability, the analytical balances used shall be calibrated with weights traceable to the national/international standards.

~~15.~~14. Calibrated class A glassware or appropriate electronic/automatic pipettes traceable to national/international standards shall be used for volumetric measurements.

~~16.~~15. The instrumentation used in purity tests should have comparable or greater sensitivity/specificity to those used in the reference material document of the RM.

~~17.~~16. According to the reference material document, the equipment used for storing and monitoring RMs should be traceable to national/international standards.

~~18.~~17. ISO Standard 33405~~Guide~~[v] may be referred for assessing the shelf-life of an RM

~~19.~~18. To ensure the validity of the stability and purity testing protocols provided below, ~~the~~ gravimetric records shall be maintained for RMs (opened or unopened), both solid and liquid, and their respective stock solutions during storage before and after use at each time. Before recording the weight, the container should attain room temperature/ambient temperature and be wiped to remove any adhering moisture. The exposure of RM and stock solutions to ambient temperatures and light must be kept as short as absolutely necessary, limiting handling time~~.~~

~~20.~~19. The record of the storage conditions (e.g., temperature and humidity) as well as the date of use of the RM and their stock solutions shall be maintained. Also, the temperature at which the RMs and their stock solutions are opened for use shall be recorded. Data loggers and control charts shall be maintained by laboratories to document and evaluate the performance of storage equipment over time.

~~CRITERIA FOR~~RECOMMENDED STORAGE CONDITIONS FOR PESTICIDE REFERENCE MATERIALS AND THEIR STOCK SOLUTIONS

20. The storage conditions of RMs are specified by RMPs in the reference material documents, as ~~these~~ RMs are susceptible to degradation at high temperatures and other unfavorable environmental conditions. Environmental conditions (temperature and humidity, as appropriate) shall be recorded, monitored and controlled by the laboratory.

21. If a laboratory maintains the RMs at ~~better~~ storage ~~conditions, i.e.,~~conditions more protective than those recommended by the RMPs (i.e., temperature lower than recommended without exposure to light and moisture, duration of handling, frequency of use etc.), the rate of degradation of the RMs is significantly minimized as long as these conditions do not contradict those indicated in the reference material document by the RMP. Under such conditions, the expiry date as recommended by the RMPs may be extended as appropriate for an RM by a date allowing for storage of up to 10 years or as long as the purity mentioned in the reference material document holds good (≤ ±10%) (~~SANTE[5]~~SANTE[vi], 2024). Another study revealed the stability of pesticide reference standards for up to 15 years or in-stock solutions for up to 10 years[i,ii].

22. To avoid any cross-contamination or degradation of RMs, the vials ~~can~~ may be placed in an airtight capped tube/sealed pouch (made of suitable polypropylene or high-quality plastic material) and immediately stored in the freezer/refrigerator at conditions more protective than those recommended by RMPs, preferably at subzero temperature. The stock solutions must also be stored in airtight capped glassware or any other suitable ~~material~~ type of vessels as specified by the RMP. Storage conditions shall be monitored, controlled and recorded ~~monitored~~ with appropriately calibrated equipment ~~and controlled and recorded~~. Exposing glassware to ~~extreme~~ elevated temperatures should be avoided.

ANALYTICAL PROTOCOL FOR MONITORING THE STABILITY AND PURITY OF PESTICIDE REFERENCE MATERIALS AND INDIVIDUAL STOCK SOLUTIONS

23. Three analytical approaches may be considered for monitoring the stability and purity of individual RMs, ~~and their~~ RM stock solutions, and mixed pesticide standard solutions of RMs ~~and mixed pesticide RM standards and~~ for the purpose of extending their use beyond the expiry date, provided their purity is proven acceptable.

24. In Approach 1, the stability of new (or unexpired) and old (or expired) RMs is determined simultaneously, ~~and it is applicable for individual neat standards and their related stock solutions.~~ The comparisons of peak area shall be ~~run under repeatability conditions and mitigate~~based on averaged values from repeated runs, which mitigates other sources of variation in instrument response~~, by averaging the values of replicate measurements~~. Alternatively, an internal standard (IS) may be used to compare the peak area ratio of new (or unexpired) and old (or expired) RMs. If the deviation (in peak area) after expiration date indicted by RMP is found within ±10%, or alternatively the peak area ratio deviation is within ±10%, the analyte in the old (or expired) RM is considered at an acceptable level and ~~and, therefore, can~~ may therefore continue to be ~~considered for continued~~ used as a valid RM. For neat standards and stock solutions, monitoring of stability & purity may be continued regularly up to a maximum of 10 years (SANTE) provided the deviation in purity from the original RMP remains ~~acceptable[1]~~acceptable[i,2ii,6vii]. Here, a new (or unexpired) RM would be required ~~for~~ throughout the period of comparison. Approach 1 is applicable for individual neat standards and their related stock solutions.

25. In Approach 2, whenever a new (or unexpired) RM is procured by any laboratory, its purity is monitored periodically before and after expiry using the same analytical conditions as mentioned in the reference material document. Here, new (or unexpired) RM need not be procured. An unexpired IS of any pesticide RM, appropriate for the method is used to account for any change in the response of the equipment. ~~This approach applies only to neat RMs accompanied by reference material documents.~~ As the analyte is spiked with the IS, the selection of the IS should be based on previous experience that shows a good stability over the expected storage time. The IS should ~~further show a good measurement behavior~~ not be susceptible to chemical degradation, ~~should~~ be insensitive to external factors such as light and moisture, chemically different from the ~~analyte~~analyte and should not interfere with the measurement of the tested analyte. ~~This approach applies only to neat RMs~~ accompanied by reference material documents.

26. In Approach 3 ~~is similar to Approach 1 wherein the stability of new (or unexpired) and old (or expired) RM is determined simultaneously and is applicable to mixed pesticide~~ standard ~~RM standards~~ solutions, three different methods have been proposed to monitor the purity of mixed pesticide standard solutions. ~~As i~~In ~~approach~~ method 1, the comparisons of peak area of each pesticide RM in new (or unexpired) and old (or expired) mixture ~~in the mixture~~ shall be ~~run under repeatability conditions and mitigate~~ based on averaged values from repeated runs, which mitigates other sources of variation in instrument response, by averaging the values of replicate measurements. ~~Alternatively~~In the method 2, an ~~internal standard (~~IS~~)~~ may be used to compare the peak area ratio of each RM pesticide in new (or unexpired) and old (or expired) mixture. If the deviation (in peak area) after expiration is found within ±10% (Method 1), or ~~alternatively~~ the peak area ratio deviation is within ±10% (Method 2), for each pesticide RM in the mixture, the analyte in the RM is considered at an acceptable level ~~and, therefore~~, ~~can~~ and may therefore continue to be ~~considered for continued~~ used as a valid RM. In method 3, whenever a laboratory acquires a new (or unexpired) mixed pesticide standard ~~RM~~from an RMP, its signal stability should be periodically monitored before and after the expiration date, employing analytical conditions as similar as possible to those indicated in the reference material documentation. In this case, it is not necessary to acquire a new (or unexpired) ~~RM~~ mixed pesticide standard from an RMP. Instead, an unexpired ~~internal standard (~~IS~~)~~, corresponding to any suitable pesticide RM for the method, is used to compensate for possible variations in instrumental response. In this procedure, the analyte peak area (or alternatively, the peak area ratio) for every pesticide in the mixture is plotted against storage time. Subsequently, linear regression analysis is applied to assess the presence of significant changes in the analyte area (or area ratio), in accordance with the classical stability study approach established in ISO 33405. If the regression analysis determines that the data do not show a linear trend (slope close to zero), the analyte contained in the RM is considered acceptable and may continue to be used as a reference material. If any of the components do not meet the ±10% criterion, the mixed pesticide standard solution may not be used as an RM. However, it may be noted that only limited publications on the behaviour of the pesticides~~by~~ ~~laboratories that behavior of pesticides~~ in mixtures over long term storage are available at this time ~~has not been extensively studied~~ and ~~that~~ caution should be taken when proposing to extend the life of an expired RM mixture. Mixed pesticide standard~~s~~ should be used for a limited time period and the stability of the analytes contained need to be demonstrated under conditions reflecting their routine use.

Approach 1: Comparing the stability of old (or expired) and new (or unexpired) pesticide reference standards~~:~~ (applicable to neat standards of reference materials and related stock solutions~~)~~

27. Prepare a fresh stock solution of the old (or expired) and new (or unexpired) RM standard of the appropriate concentration~~. Appropriate concentration will~~ depending on the response of the RM in the detector. Generally, for HPLC[1]-DAD[2]/GC[3]-FID[4], a good response is obtained between ~~10~~ 5 mg L^{-1} to 100 mg L^{-1}. For single quadrupole GC-MS[5] or LC[6]-MS, or other mass spectrometry methods, the appropriate concentration typically ranges from ~~1~~0.5 to 5 mg L^{-1}, while for triple quadrupole GC-MS/MS or LC-MS/MS, 0.1 to 0.5 mg L^{-1} or lower concentration may be more appropriate to avoid signal saturation.

~~21.~~28. Analyze the standard solution of the old (or expired) and new (or unexpired) RM on a proper instrument (HPLC-DAD, HPLC-UV[7], GC-FID, LC-MS or GC-MS, LC-MS/MS, GC-MS/MS, or qNMR[8]) and record the peak area. Either of the two methods described below ~~can~~ may be employed.

~~22.~~29. Method 1 (Peak Area Comparison): Inject standard solutions of the old (or expired) and new (or unexpired) individual RMs prepared from the stock solution at the same concentration into the instrument and record the peak area. It is recommended that the injection sequence contains at least five replicates of old (or expired) and new (or unexpired) ~~and old (or expired)~~ standards and should be alternating to minimize the impact of drifting of signal response in the course of measurement. Calculate the mean value of the peak area for the old (or expired) and new (or unexpired) RM of the five replicates. The %RSD of the replicate measurements should be ≤ ±10%. Calculate the % deviation in average peak area of the old (or expired) and new (or unexpired) standard solutions using the formula below given. The mean value from the new (or unexpired) solution is taken to be 100% and is also used as a basis for calculating the percentage difference.

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1 High-performance liquid chromatography
2 Diode-Array Detection
3 Gas chromatography
4 Flame ionization Detector
5 Mass Spectrometry
6 Liquid Chromatography
7 Ultra-violet spectroscopy
8 Quantitative Nuclear Magnetic Resonance

$$\% \ deviation = \frac{\left|(Mean\ peak\ area\ for\ old\ (or\ expired)\ standard\ - Mean\ peak\ area\ for\ new\ (or\ unexpired)\ standard)\right|}{Mean\ peak\ area\ for\ new\ (or\ unexpired)\ standard}\ x\ 100$$

~~23.~~30. Method 2 (Peak Area Ratio Comparison): Spike ~~a different~~another RM (inert and unexpired) as an IS into the standard solutions of the old (or expired) and new (or unexpired) RMs prepared from the stock solution at the same concentration. Inject the solutions and record the peak area of the RM and the IS, perform a minimum of five replicate measurements of old (or expired) and new (or unexpired) ~~and old (or expired)~~ standards ~~and~~ that should be alternated to minimize the impact of drifting of signal response in the course of measurement., and calculate the average ratio of RM area to IS area for the old (or expired) and new (or unexpired) RMs with %RSD ≤ 10%. The IS peak should have a similar abundance to the RM being verified, and it should not interfere with the analysis of the target RM in terms of either retention time or molecular weight (m/z). Calculate the % deviation using the below given formula:

$$\% \ deviation = \frac{\left|(Mean\ peak\ area\ ratio\ of\ old\ (or\ expired)\ and\ internal\ standard\ - Mean\ peak\ area\ ratio\ of\ new\ (or\ unexpired)\ and\ internal\ standard)\right|}{Mean\ peak\ area\ ratio\ of\ new\ (or\ unexpired) and\ internal\ standard}\ x\ 100$$

~~24.~~31. If the % deviation (as obtained from the above Method 1 or Method 2) shows a deviation of ≤ ±10%, the old (or expired) standard may be considered suitable for continuing use.

~~25.~~32. The old (or expired) standard shall be compared with the new (or unexpired) standard at regular intervals of at least once a year, provided the recommended storage conditions are maintained.

~~26.~~33. To monitor the stability of the RM over time, a plot of the % deviation vs. time of monitoring may be made, which would help identify the deviation in stability of RM with time.

Approach 2: Verification of purity of neat standards of pesticide reference materials during prolonged storage (not suitable for verification of stock solutions)

~~27.~~34. To verify the purity of the RM, a chromatographic assay shall be performed, preferably as per the analytical conditions mentioned in the reference material document by the RMP, with the capability of resolving and detecting the target analytes away from all of its potential impurities. If it is not feasible to match the exact conditions of the RMP, deviations should be documented and justified. Furthermore, if the deviation comes from the use of a different technique, the laboratory must guarantee that the technique has an equivalent or better sensitivity and specificity. RM purity is verified by comparing the purity (in terms of percent peak area) obtained through analysis with the purity mentioned in the reference material document.

~~28.~~35. Prepare a fresh stock solution of the new (or unexpired) neat standards of RMs and IS (a different unexpired RM) of appropriate concentration in a suitable solvent. The IS solution should be prepared in the same solvent in which the stock solution is prepared to consider any background interference that may be present. Appropriate concentration will depend on the response of the RM using the selected detection method~~; please~~ (see paragraph 2~~7~~67 of Approach 1 for suggested concentration ranges).

~~29.~~36. Prepare the standard solution of the RM from the stock solution and analyze it through the instrument (HPLC-DAD, HPLC-UV, GC-FID, LC-MS, GC-MS or other mass spectrometry methods in full scan mode, or qNMR) as per the analytical conditions mentioned in the reference material document. The percentage of peak area obtained through the software of the instrument ~~while carrying out the analysis~~ is recorded as ~~percent~~ purity. Inject a blank solution of the same solvent in which the stock solution is prepared prior to this to ~~consider~~ check any background interference that may be present. A minimum of five replicate measurements shall be performed to obtain a mean value of ~~percent~~ purity, and the %RSD of the replicates should be ≤ 10%. The instrument shall be calibrated as per the ~~conditions~~ procedures and criteria recommended by the manufacturer.

~~28.~~37. Compare the mean value of verified purity (percent purity) obtained from the laboratory analysis with the reference value of purity provided in the reference material document. The certified value (reference value) listed in the reference material document is considered as the purity reference value while calculating % deviation in purity.

~~30.~~38. The % deviation in percent purity ~~can~~ shall be calculated as:

$$\%\ deviation = \frac{\left|(\text{~~Mean peak percent area for~~Average percent area of the peak of ~~neat~~ standard} - \%\ Purity\ reference\ value)\right|}{\%\ Purity\ reference\ value}\ x\ 100$$

~~31.~~39. To determine changes in the response of the equipment with time, spike the solution of an unexpired IS of the same concentration as RM in the standard solution of RM. Inject the solution and record the peak area of the RM and the IS and calculate the average ratio of the RM area to the IS area. The IS peak should have a similar abundance to the RM being verified, and it should not interfere with the analysis of the target RM in terms of either retention time or molecular weight (m/z). Monitoring the signal of the RM with respect to IS helps to take into account the signals that may not be visible but contribute to the percent share of the analyte on the summed area of the chromatogram.

~~32.~~40. Repeat the same procedure at regular intervals of at least once a year using a freshly prepared solution of the RM and compare with the freshly prepared solution of the unexpired IS, particularly before and after the RM's expiry, to monitor its stability and purity during prolonged storage and obtain % deviation in the ratio of peak area.

$$\%\ deviation = \frac{\left|(Mean\ peak\ area\ ratio\ of\ RM\ \text{~~before~~}after\ expiry\ and\ IS - Mean\ peak\ area\ ratio\ of\ RM\ \text{~~after~~}before\ expiry\ and\ IS\right|}{Mean\ peak\ area\ ratio\ of\ RM\ \text{~~after~~}before\ expiry\ and\ IS}\ x\ 100$$

~~33.~~41. After the expiry date of the RM as indicated by its RMP, if the mean value of percent purity in terms of percent peak area obtained for the RM and the reference value (as obtained from reference material document) do not differ by more than ±10% (the % deviation of less than or equal to ±10%) in approach 1 or~~and~~ the deviation (%) in the ratio of peak area for the RM compared to the ~~internal standard~~IS is ≤ ±10% in approach 2, the RM may be considered suitable for continuing use as a valid RM in the laboratory.

Approach 3: Verification of stability of mixed pesticide standard solutions ~~mixed pesticide RM standard solutions~~ during prolonged storage.

~~34.~~42. ~~This approach has been aligned with Approach 1.~~ For ~~M~~method 1 and 2, ~~P~~prepare a fresh stock solution / working solution of the new (or unexpired) and old (or expired) mixed pesticide standard solution~~mixed pesticide RM standard~~ of appropriate concentration in a suitable solvent. Appropriate concentration will depend on the response of the RM using the selected detection method~~; please~~ (see paragraph 2~~6~~7 of Approach 1 for suggested concentration ranges).

~~35.~~43. In method 1 and 2, ~~A~~analyze the standard solution of the old (or expired) and new (or unexpired) mixed pesticide standard solution ~~RM mixture~~ on an appropriate ~~proper~~ instrument (GC or LC with detectors of appropriate specificity~~HPLC-DAD, HPLC-UV, GC-FID,~~ including ~~-~~LC-MS/~~,~~ GC-MS in full scan, GC-MSMS/LC-MSMS in MRM mode or other mass spectrometry methods or qNMR) as per the analytical conditions mentioned in the reference material document and record the peak area. Either ~~of the two methods~~method 1 or 2 described below ~~can~~ may be employed.

~~36.~~44. Method 1 (Peak Area Comparison): Inject standard solutions of the old (or expired) and new (or unexpired) mixed pesticide standard solutions~~mixed pesticide RM~~ prepared from the stock solution at the same concentration into the instrument and record the peak area of each pesticide RM in the ~~mixture~~mixed pesticide standard solution. It is recommended that the injection sequence contains at least five replicates of new (or unexpired) and old (or expired) standards and should be alternating to minimize the drifting of signal response in the course of measurement. Calculate the mean value of the peak area of the five replicates for the old (or expired) and new (or unexpired) RM. The same will be calculated for all the pesticide RMs in the mixed pesticide standard solutions ~~mixture~~. The %RSD of the replicate measurements should be ⩽ 10%. Calculate the % deviation in average peak area of each pesticide RM in the old (or expired) and new (or unexpired) ~~standard solutions of the~~ mixed pesticide standard solutions ~~mixture~~ using the formula below given:

$$\%\ deviation\ (for\ each\ pesticide\ RM) = \frac{\left|(Mean\ peak\ area\ for\ old\ (or\ expired)\ standard - Mean\ peak\ area\ for\ new\ (or\ unexpired)\ standard)\right|}{Mean\ peak\ area\ for\ new\ (or\ unexpired)\ standard}\ x\ 100$$

~~37.~~45. Method 2 (Peak Area Ratio Comparison): Spike a different RM (inert and unexpired) as an IS into the standard solution of the old (or expired) and new (or unexpired) mixed pesticide standard solutions ~~mixed pesticide RM~~ prepared from the stock solution at same concentration. Inject the solutions and record the peak area of each pesticide RM in the old (or expired) and new (or unexpired) mixed pesticide standard solutions ~~mixture~~ as well as the IS by performing a minimum of five replicate measurements with %RSD ≤ 10%. Calculate the average area ratio of each pesticide RM in the old (or expired) and new (or unexpired) mixed pesticide standard solutions ~~RM mixture~~ to the IS. The IS peak should have a similar abundance to the RM being verified, and it should not interfere with the analysis of the target RM in terms of either retention time or molecular weight (m/z). Calculate the % deviation of each pesticide RM in the mixture using the below given formula:

$$\%\ deviation\ (for\ each\ pesticide\ RM) = \frac{\left|(Mean\ peak\ area\ ratio\ of\ old\ (or\ expired)\ and\ internal\ standard\ - Mean\ peak\ area\ ratio\ of\ new\ (or\ unexpired)\ and\ internal\ standard)\right|}{Mean\ peak\ area\ ratio\ of\ new\ (or\ unexpired)\ and\ internal\ standard} x\ 100$$

46. If ~~every~~ pesticide RM in the mixture (as obtained from either Method 1 or Method 2 above) shows a deviation of ≤ ±10% relative to the ~~product information sheet~~reference material document provided by the RMP, the old (or expired) mixed pesticide standard solution may be considered suitable for continuing use. If any of the RMs in the mixture (as obtained from either Method 1 or Method 2 above) shows a deviation of > ±10% relative to the ~~product information sheet~~reference material document provided by the RMP, the old (or expired) mixed pesticide standard solution is not~~NOT~~ suitable for continued use for the quantification of pesticides not meeting the required criteria. ~~and should be discarded.~~

47. Method 3: Prepare ~~A~~a fresh solution of ~~stock solution~~ mixed pesticide standards ~~should be prepared~~ from the unexpired RM ~~standard~~ and an ~~internal standard~~IS (another unexpired RM), at an appropriate concentration in a suitable solvent. The appropriate concentration will depend on the RM response in the detector. Analyze t~~T~~he mixed pesticide standard solution~~RM standard solution~~, prepared at an appropriate concentration from the stock solution, ~~should then be analyzed~~ on an appropriate ~~the~~ instrument as described in paragraph 4~~1~~3, ~~(HPLC-DAD, HPLC-UV, GC-FID, LC-MS/MS, GCMS/MS, LC-HRMS, GC-HRMS [full scan, SRM or MRM mode], or qNMR),~~ following analytical conditions ~~similar~~as close as possible to those established in the reference material document~~ation~~. Perform a~~A~~t least five replicate measurements ~~should be performed~~ to obtain a mean value of the ratio between the RM area and the ~~internal standard~~IS area for each individual component. To verify measurement reproducibility, the %RSD of the replicates should be ≤ 10%. Repeat t~~T~~he same procedure ~~should be repeated~~ at regular intervals of at least twice a year, using a new solution of mixed pesticide standards~~RM stock solution~~, particularly before and after the expiration date, in order to monitor its stability and purity during long-term storage. Once the RM expiration date has been reached, repeat the procedure ~~should be repeated~~, and prepare a plot of the area or area ratio of each component versus time ~~should be prepared~~. After obtaining the data, perform a linear regression analysis ~~should be performed~~ to determine whether the model adequately fits the data obtained in accordance with the classical stability study approach established in ISO 33405. If the regression analysis indicates that the data do not show a linear trend (coefficient of determination $R^2 < 0.8$, p-value > 0.05)~~,~~ and slope close to zero~~)~~, it may be concluded that storage time has not contributed to any change in the response obtained. Consequently, RM may be considered suitable for continued laboratory use.

~~38. If the % deviation for every pesticide RM in the mixture (as obtained from the above Method 1 or Method 2) shows a deviation of ≤ ±10% relative to the product information sheet provided by the RMP, the old (or expired) mixed pesticide RM mixture~~standard solution ~~may be considered suitable for continuing use. If any of the RMs in the mixture (as obtained from the above Method 1 or Method 2) shows a deviation of > ±10% relative to the product information sheet provided by the RMP, the old (or expired) mixed pesticide standard solution is NOT suitable for continued use and should be discarded.~~

~~39.~~48. The ~~old (or expired) mixed pesticide standard solution~~mixed pesticide RM standard ~~shall be compared with the new (or unexpired) mixed pesticide standard solution mixed pesticide RM standard~~ monitoring of the stability of the mixed pesticide standard solution shall be performed at regular intervals of at least twice a year, provided the recommended storage conditions are maintained.

~~40.~~49. To monitor the stability of the mixed pesticide standard solution ~~mixed pesticide RM standard~~ over time, a plot of % deviation vs. time of monitoring may ~~be made, which would~~ help identify and predict the deviation in stability with time.

ANNEX

DEFINITIONS

Certified Reference Material (CRM): Reference material (RM) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

Internal standard: A chemical added at a known amount to samples and/or standards in chemical analysis, including the blank and calibration standards. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the concentrations. This ratio for the samples is then used to obtain the analyte concentrations. The internal standard used needs to provide a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable from each other.

Mixed pesticide standard solution~~Mixture of Reference Material~~**:** Reference standard of pesticide containing multiple compounds procured from a Reference Material Producer (RMP) accredited as per ISO 17034 or a mixture of pesticide standards prepared by the laboratory from individual RMs procured from RMPs to ensure analytical traceability or from a National Metrology Institute recognized by peers or designated by countries

Reference Material Document: A document that provides the relevant information about certified purity, concentration, date of expiry, and measurement uncertainty of an RM, which is in compliance with the requirement in ISO 17034 and ISO 33401[viii]. Reference material documents can be in the form of a Product Information Sheet or Certificate of Analysis (CoA).

Purity: Characteristic of a reference material which indicates the proportion of the stated component of interest in relation to the total substance. Purity is typically expressed in percentages and should be considered when preparing standard solutions.

Quality Control Material (QCM): Reference material used for quality control of a measurement.

Reference Material (RM): Material sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Reference Material Producer (RMP): Body (organization or company, public or private) that is fully responsible for project planning and management; assignment of, and decision on property values and relevant uncertainties; authorization of property values; and issuance of a reference material certificate or other statements for the reference materials it produces.

Relative Standard Deviation (%RSD): It is expressed as the sample standard deviation divided by the sample mean multiplied by 100.

Stability: Characteristic of a reference material, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period of time.

Standard solution: A chemical solution that has a precisely known concentration. Standard solutions are generally prepared by dissolving a solute of known mass into a solvent to a precise volume or by diluting a solution of known concentration with more solvent.

Stock Solution: A solution of a reference material or standard of high concentration from which appropriate dilutions can be made at the time of use.

Traceability: Metrological traceability, property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

Uncertainty: measurement uncertainty, non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.

Reference Documents

i. de Kok, A., de Kroon, M. and Kiedrowska, B. (PO 005 pdf, 2019). Stability of pesticides reference standards and stock solutions Part 1 GC-pesticides NVWA - Netherlands Food and Consumer Product Safety Authority, Laboratory of Food and Feed Safety-Chemistry Laboratory, National Reference Laboratory (NRL) for Pesticide Residues in Food and Feed, Wageningen, The Netherlands.

ii. de Kok, A., de Kroon, M. and Scholten, J. (PO 006 pdf, 2019). Stability of pesticides reference standards and stock solutions Part 2. LC-pesticides NVWA - Netherlands Food and Consumer Product Safety Authority, Laboratory of Food and Feed Safety-Chemistry Laboratory, National Reference Laboratory (NRL) for Pesticide Residues in Food and Feed, Wageningen, The Netherlands.

iii. Sharma, K. K., Tripathy, V., Gautam, R., Gupta, R., Tayade, A., Sharma, K., Yadav, R., Shukla, P., Devi, S., Pandey, P., Singh, G., Kalra, S., Walia, S. (2020). Monitoring of purity of CRMs of multi-class pesticides during prolonged storage before and after expiration. Accreditation Qual. Assur., 25 (10), 89-97. 10.1007/s00769-019-01411-w.

iv. ISO/IEC 17025: 2017- General requirements for the competence of testing and calibration laboratories

v. ISO 33405:2024-Reference Materials- Approaches for characterization and assessment of homogeneity and stability

vi. SANTE/11312/2021 V2, Implemented by 01/01/2024, European Commission Directorate General for Health and Food Safety.

vii. EURL DataPool, https://www.eurl-pesticides-datapool.eu/

viii. ISO 33401:2024- Reference materials — Contents of certificates, labels and accompanying documentation