Stabilization strategies for unstable pesticides in calibration reference materials

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Abstract – **To achieve measuring methods harmonization, improve reliability, and quality of measurement results, the use of certified reference materials (CRM) are necessary. Due to the instability of some compounds, the offer of CRM of pesticides is low. The National Metrology Institute of Colombia, as reference materials producer, has evaluated strategies to determinate and improve the stability of ten multiclass selected pesticides in calibration solutions. For this purpose, to obtain an overview of the degradation behavior of pesticides, ten compounds with different physicochemical characteristics were chosen. Three mixtures were prepared, which contained one polychlorinated biphenyl (PCB) as control. Various strategies were applied for the stabilization of the pesticides (an additive, inert atmosphere, and their combinations). The stability assessment was performed using an isochronous design at extreme conditions to induce a faster degradation of the compounds. Three analytical methods using gas chromatography were developed and validated. After methods validation, the stability measures were realized under repeatability conditions. The results were evaluated using regression. The results demonstrated that the strategies used for stability increasing were adequate for all of the evaluated compounds. In this study, it was found that when a stability strategy is adopted, the uncertainty associated with the stability improve up to 15 times.**

Keywords – Pesticide, Stability, Reference Materials, Uncertainty, Method validation.

I. INTRODUCTION

Pesticides are chemicals compounds specifically developed and produced for use in the control of agricultural and public health pests. They have emerged as one of the most powerful tools of pest management to have food security [1].

Due to its toxicity, different regulations for its use and application have been established worldwide. In this sense, the agrochemical producers should be carrying out strict quality controls during the production process and their formulations, through the implementation of analytical methodologies, to ensure that the inputs used in the production processes and the finished products comply with the legally established requirements.

The agrochemical producers and, in general, the analytical laboratories should be using different quality assurance tools to provide objective proof of their technical competence and reliability of results. This need stems from the fact that many technical, economic, and political decisions are based on the results of analytical measurements. For this reason, it is necessary to ensure an adequate system of quality and assurance control of the results of analytical measurement; the reference materials (RM) play a key role in achieving this aim.

An RM has been defined by ISO 30 [2] as a material, sufficiently homogeneous and stable concerning one or more specified properties, which has been established to be fit for its intended use in a measurement process. Taking into account the above, comprehensive studies have to perform, employing methods with high repeatability to detect possible inhomogeneity and instability of the material. However, the material stability could be representing a challenge to reference material producers (RMP) due to some compounds are susceptible to suffering undesirable reactions by environmental or physicochemical factors causing changes on the property value during storage or material production, involve short term, extremely storage conditions, or preventing its certification. This situation is reflected in the low commercial offer of RM for some analytes [3].

An example of unstable analytes are some pesticides, which have been reported as unstable because they could be sensitive to degradation by temperature, light, pH, microbial activity, etc. [4], decreasing the possibility for RMP to certificate the property values.

Considering the above, the objective of this work is to evaluate different strategies as additives and inert atmosphere or their combinations to increasing pesticide

stability in calibration solutions. In this sense, three analytical methods were developed and validated. After method validation, the stability studies were performed, and the uncertainty due to the stability of the materials was calculated to evaluate the efficiency of the strategies applied.

II.   RELATED RESULTS IN THE LITERATURE

The instability of some pesticides has been a challenge in different areas of analytical chemistry. In this sense, several investigations around their stability have been performed. Ferrer et al. [5] evaluated the use of polymeric solid-phase extraction cartridge to storage instable pesticides as desethylatrazine, fenamiphos, fenitrothion, and fonofos, which are present in water samples. This study has demonstrated an improvement in the stability of fenamiphos and fonofos, stored at 4 ºC for a week and one month, respectively. In the same way, that study demonstrated the acidification and the addition of biological inhibitors as $HgCl_2$ has a negative effect in many cases as regards the stability of pesticides in water since many of them become hydrolyzed or degraded. The best storage conditions for all the pesticides on polymeric cartridge were those corresponding to storage at -20°C since no losses and no interfering peaks were achieved after one month of storage.

On the other hand, Maštovská et al. [6] investigated the pesticide stability in common organic solvents used for extraction and pesticide analysis by gas chromatographic to select the solvent suitability and improve the stability of compounds during sample kept in the autosampler (max. 5 days). Certain pesticides (dicofol, chlorothalonil, captan, folpet, and dichlofluanid) are degraded rapidly in acetonitrile. Therefore, it attempted to stabilize them by adding acetic acid (HAc) to the standard solutions. Founding the stability of these analytes as well as of dicofol and chlorothalonil was dramatically improved by the addition of 0.1% HAc (v/v) to MeCN. Dicofol and chlorothalonil were also unstable in acetone, but the addition of 0.1% HAc did not sufficiently suppress degradation in this case. They concluded that after acidification, the stability of problematic pesticides in MeCN was acceptable.

Guo et al. [7] studied the storage stability of three organophosphorus pesticides, phorate, disulfoton, and fenthion, encountered that the stability of these compounds was affected by the type of solvent, light, and temperature; degrading quickly, especially in pure ethyl acetate solvent. In ethyl acetate, all three pesticides decreased more remarkable than 30% when stored for three days at −20°C. For a disulfoton pesticide, light played a substantial effect on stability due to its structure and its physicochemical properties. Also, these findings provided additional information that temperature could significantly affect storage stability. It was concluded that even at −20°C, exposure to solvents or matrix extracted solution resulted

in a significant loss. Therefore, it could be assumed that the optimal storage conditions for these three organophosphorus pesticides include a lower temperature, storage in a brown vial, and using acetone or hexane as solvents.

Respect to reference materials, several works have been reported for the use of different strategies to improve pesticide stability. However, the majority of these studies are addressed to matrix reference materials. In general, usual strategies for increasing the stability of pesticides in matrix materials include drying of the matrix through freeze-dried, use of brown bottled to avoid light exposure, antioxidants addition, and lowest storage temperatures [7]–[11].

III.   DESCRIPTION OF THE METHOD

A.   Standards and reagents

Pesticides and PCB used in this study were obtained as analytical standards from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Calibration solutions were gravimetrically prepared using acetonitrile and ethyl acetate as solvents (HPLC grade) obtain from Merck (Darmstadt, Germany).

B.   Instrumentation

The pesticide determination was performed using a 7890 series gas chromatograph, equipped with a micro electron capture detector ($\mu ECD^{63}Ni$) and mass-selective detector (MSD) 5975C Agilent Technologies (Palo Alto, CA, USA). The MSD was equipped with an electronic impact source, operated at 250 ºC. An HP-5ms column from J&W Agilent (30m×0.25mm×250 um) was used. A split/splitless injector worked in split mode was used. The carrier gas was helium (99.9995% purity) and was operated at a constant pressure rate. The makeup gas for the $\mu ECD^{63}Ni$ detector was nitrogen. The instrument control and results analysis were realized by MassHunter software.

C.   Preparation of candidate RM

The ten pesticides used for the preparation of the materials were grouped in three mixtures, consisted of three or four pesticides reported as unstable [12] and a very stable compound (polychlorinated biphenyl, PCB-138). The candidates were gravimetrically prepared using acetonitrile as solvent. After preparation, the materials were shaken for one hour. The materials were divided into applicate stabilization strategies, bottled in glass ampoules, and finally, the units were sealed using an oxygen/acetylene flame.

D.   Stabilization strategies

Different treatments were evaluated to study pesticide stability in solution. Each mixture was divided into three portions to applicate: (i) inert atmosphere, for this each ampoule was saturated with argon to decreasing the

oxidation possibility for the test compounds; (ii) formic acid (0.1%, p/p) and argon atmosphere was used as a second treatment, for this purpose a portion of each mixture was added with formic acid to avoid the pesticide degradation due to pH, and finally was saturated with argon before sealed, and (iii) the mixtures were bottled in a bigger ampoule, with the aim to increasing the oxidation environmental.

E. Analytical methodologies

Pesticide determination was performed by GC-MSD and GC-μECD. Three analytical methods were developed. In all the instrumental methods, 1 uL of the sample was injected in a split/splitless injector operated in split mode. The method validations were carried out for the most relevant parameters in this study. The parameters evaluated were selectivity, linearity, and precision. For the sample preparation, each mixture was diluted with ethyl acetate.

For assessing methods selectivity, blanks solvents samples (ethyl acetate and acetonitrile), each pesticide was injected separately, and the mixture without each compound was analyzed and checked for the absence of interfering peaks at the retention time of pesticides. The methods linearity was studied by injecting thirteen concentration levels gravimetrically prepared from 1 mg/kg to 30 mg/kg. Each subsample was injected seven times on the GC. To assess the method precision as repeatability, three concentration levels were evaluated. Each subsample was injected seven times on the GC. The results were expressed as RSD, and the homogeneity of the variances was tested using the Levene's test.

F. Stability assessment

The stability study was carried out following the stipulated in ISO 35 [13]. An accelerated isochronous design was used. All materials were exposed to accelerated conditions (50 ºC) for 59 days. At the selected time (0,14,25, 35, and 59 days), ampoules of each material and treatment were stored at reference temperature (-80 ºC). At the end of the study, all samples were diluted using ethyl acetate, and the internal standard was added. Finally, the samples were measured, under repeatability conditions, seven times in the GC instrument. The stability of the materials was evaluated by ANOVA test of the linear regression. The uncertainty due to materials stability was calculated according to ISO 35.

IV.   RESULTS AND DISCUSSIONS

A. Preparation of candidates of RMs

The material mixtures were gravimetrically prepared. The composition of each mix is presented in Table 1. Each mix contains a PCB-138 as control. In all mixtures, the pesticide concentrations were close to 50 mg/kg, and the control concentration was close to 20 mg/kg.

Table 1.Unstable pesticides evaluated

Mixture	Pesticide	Chemical group
1	Chlorpyrifos	Organophosphorous
	Folpet	Phthalimide
	Cyhalothrin, lambda	Pyrethroid
	Tebuconazole	Triazole
2	Carbofuran	Carbamate, N-methyl
	Dimethomorph	Morpholine
	DDE	Organochlorine
	Dimethoate	Organophosphorous
3	Difenoconazole	Triazole
	Chlordane, cis-	organochlorine
	Bifenthrin	Pyrethroid

B. Method development and validation

Three analytical methods were developed and validated. Chromatographic parameters as injection temperature, column pressure, split ratio, split pulse, time of the pulse, μECD temperature, and μECD gas maker flow were evaluated to obtain the lowest RSD.

The method selectivity was evaluated by the analysis of solvents blanks (ethyl acetate and acetonitrile) and the analysis of each mixture without one compound evaluated. No peaks from the solvent or other compounds present in the materials interfering with the target analytes were found. Retention time, quantification and, qualification ions were used for identification of the target analytes, which were clearly distinguished from the solvent and other compounds in the mixture.

The method linearity was evaluated according to previously described. The results of the statistical parameters calculated from least-square regression showed that determination coefficients (R^2) were higher than 0.99 for all compounds and the p-values for the lack-of-fit test ($\alpha = 0.05$) higher than 0.05, thus confirming the linearity of the target pesticides over the selected interval. Student's t statistics were used, checking that in all cases, the intercept is not significantly different from zero, and the slope is significantly different from zero.

The Levene's test results showed a homoscedastic variance for all pesticides, except to some compounds such as dimethomorph and carbofuran, which presented a higher variation in lowest concentrations. The precision of the methods evaluated as repeatability showed RSD less than 2 % for the majority of the pesticides.

According to the results of the method validation, it is possible to conclude that the proposed analytical methods are quick (between 7 min and 14 min), precise (RSD < 2%), and, selective and therefore could be appropriately applied for analytical purpose in the evaluation of the

stability of pesticide solutions.

C. Preliminary analysis

Fig. 1. shows the ability of the GC method to separate and detected some degradation products of lambda-cyhalothrin (presumably a cyhalothrin isomer). According to other investigations [6], this phenomenon occurs during the GC injection process rather than in the solutions. However, in this study, it was found that the instability of lambda-cyhalothrin occurs in the solutions and not during the GC injection; this conclusion is supported by the fact that the isomer was never detected in (i) solutions at time zero, which were stored at -80 °C; (ii) the control solutions, which were prepared on the day of the measurement by GC. Additionally, this isomer was detected in all chromatograms from time 14 days to the last time.
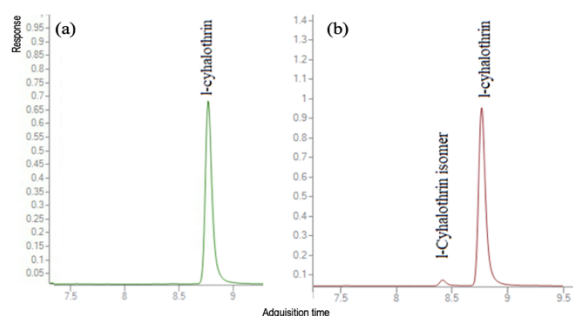

Fig. 1. Detection of the lambda-cyhalothrin isomer by the developed method (a) t=0. (b) t=14 days.

In a preliminary review of the chromatograms (Fig. 2), it was found that folpet showed a quick degradation in the materials exposed at oxidative medium and in an inert atmosphere. The folpet signals were only detectable in the ampoules (t=0) stored at the reference temperature (-80ºC) (Fig.2).
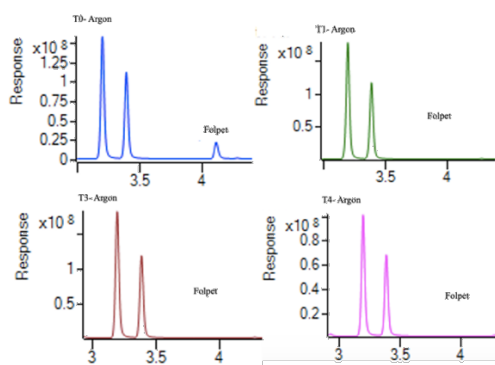

Fig. 2. Variation in the chromatographic signal of Folpet. Inert atmosphere

D. Stability Assessment

In this study, it was found that half of the examined pesticides were stable in acetonitrile at least 59 days if stored at temperatures below 50 ºC. However, pesticides

such as lambda-cyhalothrin, tebuconazole, folpet, dimethoate, and chlorpyrifos were unstable.

Concentration of pesticides in the ampoules from the stability studies were calculated as a percentage of the initial concentration. Fig. 3-5 shows the results of the stability of the three pesticides. These figures corroborate the stability and degradation problems for folpet and lambda-cyhalothrin exist.

As can be seen in Fig. 3, the addition of formic acid and the accompanying pH reduction had a significant effect on the stability of folpet in acetonitrile, considering that folpet had the same instability with only an inert atmosphere. For folpet, it is known that in an alkaline medium, the hydrolysis of the ester group gives rise to a metabolite [14]. Consequently, the addition of formic acid prevents its hydrolysis.
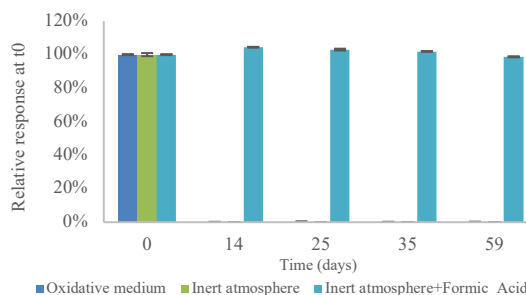

Fig. 3. Effect of the different treatments on the stability of Folpet calibration solution

Fig. 4 and Fig. 5 shows that lambda-cyhalothrin and dimethoate were unstable under oxidative conditions. In this study, it was found that the use of an argon atmosphere stabilizes these pesticides. Furthermore, as can be seen in Fig. 4. The use of formic acid did not represent any improvement compared to the use of only the inert atmosphere. Based on our results, it is feasible to suggest that possibly the instabilities of these pesticides are due to oxidation reactions.
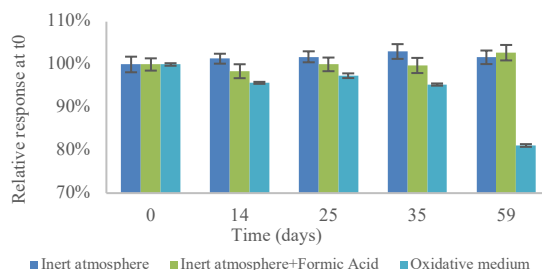

Fig. 4. Effect of the different treatments on the stability of lambda-cyhalothrin calibration solution
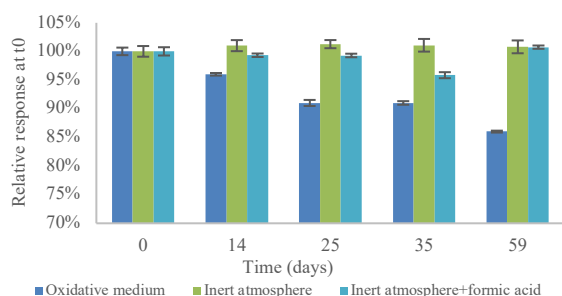
Fig. 5. Effect of the different treatments on the stability of dimethoate calibration solution

The order of degradation was determined by a graphical method. From Fig. 6 and Fig. 7 it was found that the correlation coefficient for the first-order reaction is more reliable as compared to the zero-order. Hence the degradation of dimethoate follows a first-order reaction.
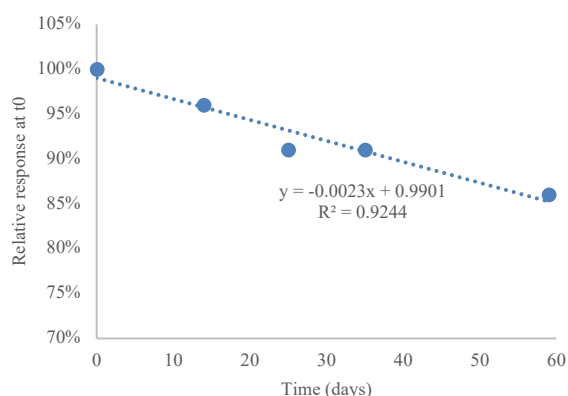

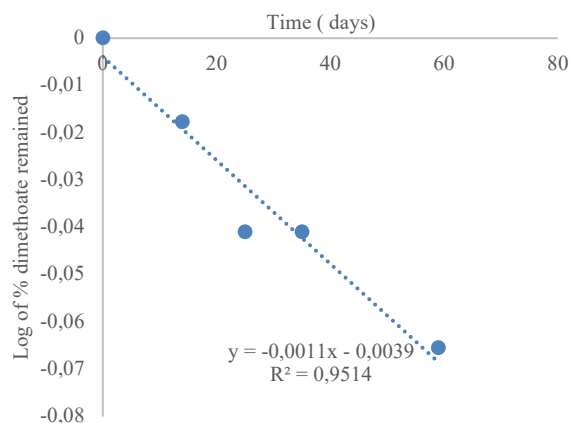*Fig. 6. Zero-order degradation kinetics of dimethoate at 50 ℃ and oxidative conditions*

Fig. 7. Fist-order degradation kinetics of dimethoate at 50 ℃ and oxidative conditions

For the remaining pesticides, the parameters and probabilities given in Table 4 indicated that the release kinetics of these pesticides followed zero-order (p-value was below 0.05). On the other hand, exposure to the oxidative medium showed an important effect on the stability of chlorpyrifos and cyhalothrin. The regression analysis results for these compounds ($\propto=0.05$) indicated Zero-order degradation kinetics. These results are in agreement with those reported by Lehotay et al. [6]

On the other hand, the estimation of uncertainty due to instability (60 days) was carried out following the ISO 35 guide. Only for dimethoate, for the first-order degradation, the log % of pesticide remaining was plotted against time (Fig. 1), and k was calculated from the slope (m) following the equation (1).

$$-m_{slope} = \frac{k}{2.303} \qquad (1)$$

Where k is the degradation rate constant, which was used to estimate the instability uncertainty (u_{stab}).

As can be seen in Table 2, the use of an inert atmosphere using argon has demonstrated its ability to maintain the stability of the chlorpyrifos, dimethoate, lambda-cyhalothrin, and tebuconazole exposed at 50 ℃ for two-months. Furthermore, the addition of formic acid (0.1 % p/p), has been successfully applied strategies to avoid the degradation and the stability of folpet.

Table 2. p-value R^2 and instability uncertainty (u_{stab}) for pesticides that exhibited some type of decomposition

Compound	Treatment	p-value	R^2	u_{stab} (%)
Chlorpyrifos	OM	0.037	0.779	16.20
	IA	0.948	0.002	1.20
	IA+FA	0.658	0.074	1.20
Dimethoate*	OM	0.007	0.936	15.00
	IA	0.442	0.206	0.60
	IA+FA	0.990	<0.01	3.00
l-cyhalothrin	OM	0.037	0.811	18.00
	IA	0.266	0.383	1.20
	IA+FA	0.135	0.580	1.80
Tebuconazole	OM	0.046	0.783	25.20
	IA	0.573	0.117	1.20
	IA+FA	0.706	0.054	3.60
Folpet	OM	-	-	-
	IA	-	-	-
	IA+FA	0.940	0.016	1.20

OM: Oxidative medium, IA: inert atmosphere,
IA+ FA: inert atmosphere+ formic acid.
First-order kinetics are presented

V.   CONCLUSIONS AND OUTLOOK

The methodologies described in this paper allowed the simultaneous determination of pesticide calibration solutions by GC-MSD and GC-µECD. These methods allow a good chromatographic separation and quantification of pesticides of different physicochemical characteristics. Coefficients of determination (>0.997) derived from calibration curves represent good linearity of the methods. According to the above, it is concluded that the methods are adequate for stability studies in reference materials.

Using a highly sensitive and precise GC assay, additional evidence has been given for the chemical instability of chlorpyrifos, lambda-cyhalothrin, tebuconazole, folpet, and dimethoate in solution, particularly acetonitrile. In acetonitrile acidified solution over severe conditions (temperatures close to 50 ºC), all the pesticides were stables until 60 days.

The use of an inert atmosphere using argon has demonstrated its ability to maintain the stability of some compounds exposed at 50 ºC for two-months. The stability uncertainties were 15 times lowest than for the compound exposure at the same condition without argon. Finally, the addition of formic acid (0.1 %) has been successfully applied strategies to avoid the degradation and the stability of folpet.

VI.   ACKNOWLEDGMENTS

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