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Establishing Laboratory Analysis Procedure for EPA FIFRA Enforcement Samples to Meet the ISO/IEC 17025:2005 Requirements using Flexible Scope Approach

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Abstract

An ISO/IEC 17025:2005 accredited laboratory procedure that quantifies analytes and validates an analytical method used simultaneously was established for analyzing pesticide misuse samples. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (EPA) grants the authority over regulating pesticide use to each state. Samples for pesticide misuse cases such as drift complaints are analyzed by state laboratories (state FIFRA labs). State FIFRA labs receive samples that require quick turnaround with a variety of unique matrix/analyte combinations. The majority of the samples are analyzed by non-standard methods that are developed by the laboratory. It is impractical to pre-validate such non-standard methods. Yet, the forensic nature of pesticide misuse investigation samples demands legally defensible analytical data. The author's laboratory developed an expedited validation procedure that is conducted simultaneously with analyte quantitation by using the standard addition technique. The procedure received ISO/IEC 17025:2005 accreditation from the American Association for Laboratory Accreditation under their flexible scope policy. The developed validation protocol and associated procedural changes made to the daily operations are supported by several laboratory documents that are unique to the flexible scope option.

Keywords: ISO/IEC 17025:2005 accreditation, pesticide misuse, FIFRA, state, flexible scope policy

1. Introduction

In the United States the Federal Insecticide Fungicide, and Rodenticide Act (FIFRA) governs the use of pesticides as well as their registration, distribution, and sale. FIFRA is administered by the Environmental Protection Agency (EPA). Under FIFRA Section 26, each state has the primary enforcement responsibilities for pesticide use violations if it is determined by EPA that a state has adequate laws and regulations and a system to administer them [8].

Samples collected for pesticide misuse investigations (FIFRA enforcement samples) are analyzed by state laboratories (state FIFRA labs), and laboratory results play a significant role in the enforcement procedures. Independent oversight of the laboratory helps assure that lab results are legally and scientifically defensible and that data produced meet the requirements for its intended use. The authors laboratory at the Minnesota Department of Agriculture (the MDA lab) sought ISO 17025:2005 accreditation for the methods used to analyze FIFRA enforcement samples.

ISO 17025 are the standards produced by the International Organization for Standardization (ISO) for general testing laboratories, such as pesticide analysis laboratories. For ISO 17025 accreditation, an independent laboratory accreditation body assesses a laboratory against the ISO standards and confirms that the laboratory meets the standards. The American Association for Laboratory Accreditation (A2LA) is the accreditation body from which the MDA lab sought the accreditation.

Achieving accreditation to ISO 17025 has become the recommended practice for most regulatory laboratories in the United States [1, 5, 6]. The accreditation is typically granted for each analytical method. However, for laboratories that analyze investigative samples, it is impractical to identify the list of methods that address all potential matrices and analytes. A2LA, under their flexible scope policy, provides laboratories who are under such circumstance an option to seek accreditation on an analytical procedure that is not limited to a set of matrices and analytes.

This manuscript describes a procedure developed by the MDA lab for analyzing pesticide misuse investigation samples. The procedure meets ISO 17025 accreditation requirements under A2LA's flexible scope policy.

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2. FIFRA Enforcement Samples and Flexible Scope Policy

An analytical method (a method) for an environmental matrix such as soil and water is normally validated using a mixture of several different types of clean matrix of interest. For vegetation, however, a mixture of several types of clean vegetation is not representative of all vegetation matrices encountered. A method has to be validated for each plant type.

The MDA lab receives vegetation samples (foliage part of plants) of a wide variety of plant types on a daily basis. The vegetation samples consist of nearly 90% of the FIFRA enforcement samples at the MDA lab and the majority of them are from pesticide drift cases. These samples often result in combinations of matrices (plant types) and target analytes that have never been encountered previously, or for which no established analytical method exists. In 2015, there were 180 vegetation samples submitted from 41 different types of plants for roughly 1080 pesticide active ingredients that are registered in Minnesota. It is impractical to try to predict all the potential sample matrix and analyte combinations and validate methods prior to sample arrival.

Typically, analytical methods are developed and validated for a defined set of matrices/analytes before testing of samples. However, the unpredictable nature of FIFRA enforcement samples require analytical procedures that are not restricted to certain matrix/analyte combinations. This situation applies to A2LA's flexible scope policy. A2LA recognizes the situation as the following: "the laboratory requires flexibility in allowing for changes in the matrices within a product area (flexibility concerning object/matrix/sample) or with respect to parameters (flexibility concerning parameters/components/analytes)" [3].

Under the policy established by the MDA lab, an expedited procedure validates a method for each vegetation sample and its matrix/analyte combination. The procedure is applied to each sample as they are received. The expedited validation allowed the MDA lab to develop a procedure for pesticide residue analysis in vegetation samples that is not limited to defined set of matrix/analyte combinations.

3. Procedure

3.1. Quantitation and Simultaneous Method Validation

FIFRA enforcement samples are from cases that can have immediate human, environmental, and/or economic impacts. They require a quick turnaround for timely regulatory action, leaving no time for conducting a conventional full method validation when an incident occurs.

In the developed procedure under the flexible scope policy, the standard addition technique serves as an expedited validation of the method for a specific matrix/analyte combination for each sample, as well as the quantitation method. Therefore, the validation and quantitation is accomplished simultaneously (simultaneous validation). The use of the standard addition technique also accounts for potential recovery issues and helps produce good quality results in a timely manner.

Under this procedure the following samples are extracted and analyzed:

- The sample as received,
- The sample with level 1 spike - Matrix Standard Addition-1 (MSA-1), and
- The sample with level 2 spike (MSA-2).

The ISO 17025:2005 Standard, section 5.4.5 describes Validation of Method and 5.4.5.3 lists the two parameters to be assessed and confirmed to meet the customers' needs; *Range* and *Accuracy* [4]. The two validation parameters and associated values are evaluated as follows:

Range: MSA-1 and MSA-2 represent the approximate range of analyte concentration within which the method performs satisfactorily.

Accuracy: Accuracy is typically expressed as the percent recovery of spiked analyte on the matrix of interest. However, the standard addition technique is one of the best quantitation techniques to compensate for any analyte loss during analysis. Since the most accurate result is obtained by the technique, no spike recovery is reported.

- Limit of Detection (LOD) is determined for a sample when no analyte is detected. A2LA's check list for Quality Systems for Chemical Testing requires LOD(s) to be verified by detection of the analyte(s) in each matrix [2]. This is done by analyzing the sample spiked with the analyte at the desired level. Section 5.2 describes more detailed LOD determination procedure.
- The uncertainty of the result is expressed by the correlation coefficient of a calibration curve (R^2) produced by the standard addition technique. A curve with $R^2 \geq 0.97$ is used to quantify a residue in samples. When a curve does not meet $R^2 \geq 0.97$, the result is reported with the remark "semi-quantitative".

To help determine the optimum MSA levels, extracts of samples are quantified by an external curve as an unofficial prescreen prior to the standard addition procedure. If the prescreen detects no target residue, the sample will be applied to the LOD determination procedure.

3.2. Quality Control

Quality control procedures monitor the validity of the total analytical system in the laboratory at the time the samples were analyzed. A mixture of several different types of clean vegetation is used as the matrix. Because the matrix is a mixture of different types of vegetation, the quality controls are not the indicator of the validity of the analytical method for each sample matrix.

The following samples are analyzed at a rate of one set per analytical batch (maximum of 20 samples):

- Negative Control - Method Blank
- Positive Control - Laboratory Control Sample (LCS)

Without Accreditation (method NOT VALIDATED)	**With Accreditation** (simultaneous method validation)
External Curve	External Curve (quantify LCS)
Method Blank	Method Blank
LCS/LCSD [a]	LCS
Sample 1	Sample 1
Sample 2	MSA [b] (sample 1)
	MSA 2 (sample 1)
	Sample 2
	MSA 1 (sample 2)
	MSA 2 (sample 2)

[a] LCS/LCSD = Laboratory Control Sample/Laboratory Control Sample Duplicate
[b] MSA = Matrix Standard Addition

Table 1: Analytical batch structures, without and with accreditation.

SOP1	Quality Manual
SOP2	**Application of Gas Chromatograph/Mass Spectrometer**
SOP3	**Application of Liquid Chromatograph/Mass Spectrometer**
SOP4	**Determination of Pesticide Residues for FIFRA Investigations**
SOP5	**Flexible Scope Approach and Application of the Standard Addition Technique**
SOP6	**Method Validation Procedure for Pesticide Residue for FIFRA Investigations**
SOP7	**Determination of Method Detection Limit for Non-Routine Samples**
SOP8	**Method Development Guideline of Pesticide Residues for FIFRA Investigations**
SOP9	**Guidance for Extraction Method Selection and LCS Spiking Compounds**
SOP10	Identification Criteria of Pesticide Residues for FIFRA Investigations
SOP11	Calibration Curve Practices for Quantitation of Pesticide Residues for FIFRA Investigations
SOP12	Guidance for Using Matrix Matched Standards for Quantitation of Pesticide Residues
WI1	Work Instructions for Assigning Analysis, Log-out and Sample Preparation Procedure

Table 2: Core standard operating procedures.

Method blank assess the samples in the batch for possible contamination during the preparation and processing steps. It is reported as “clean” or “not clean”. LCS is the clean vegetation mixture spiked with select analytes at approximately 2-5 times the method quantitation limit. The LCS is quantified by an external curve and is used to evaluate the performance of the total analytical system by charting the recoveries of the spiked analytes.

Table 1 compares generalized analytical batch structures for vegetation samples: A batch following an analytical procedure without the accreditation, and a batch following the procedure established at the MDA lab under the flexible scope option.

4. Supporting Documents and Practices

To seek accreditation under the flexible scope option a laboratory is required to demonstrate its competence to A2LA by having been accredited to a method for a defined set of matrix/analyte (fixed scope option) [3]. The MDA lab has been meeting this requirement since 2011.

Other documents and practices established by the MDA lab to meet the requirements for the flexible scope option are explained in this section.

4.1. Core Standard Operating Procedures

Table 2 lists the core standard operating procedures (SOPs) that support the procedure described in section 3. Titles for the SOPs were modified for this manuscript to provide readers with a generalized concept of the core SOP functions. The bolded SOPs are unique to the flexible scope option. Other SOPs apply to both fixed scope and flexible scope approach.

Quality Manual (SOP1) describes the quality management system of the MDA lab. It outlines all quality control practices and supporting procedures as well as management and technical requirements for the accreditation. The Quality Manual demonstrates that the MDA lab has the management system that controls the flexible scope so that the procedures described in

section 3 are carried out in accordance with the accreditation requirements.

The two instrumental techniques, GC/MS and LC/MS (SOP2 and SOP3) are under the flexible scope as *Methods*. The SOPs describe the basic operation, maintenance, and functional verification of the instrument required to run each technique respectively.

The simultaneous validation using the standard addition technique is accepted under the flexible scope policy. SOP4 describes the unpredictable nature of matrix/analyte of the FIFRA enforcement samples, while demonstrating that a fixed scope is too restrictive for the FIFRA work and that a flexible scope is justified. SOP5 explains the concept of using the standard addition technique for both quantitation and validation in depth. SOP6 describes practical details of the simultaneous validation procedure such as the levels of analytes to be added to samples and parameters to be reported as validation results. SOP7 focuses on LOD determination. LOD is determined only if a sample is clean for the requested analyte. It is determined by spiking a sample with the analyte at the desired level and applying the sample to the complete analytical method to confirm its recovery. LOD for each analyte varies among vegetation samples.

Although the standard addition technique compensates for the low recovery issue, challenging matrices/analytes might require modification and/or development of extraction method prior to applying the technique to a sample. SOP8 provides guidelines for a systematic method improvement for such situation. SOP4 describes how to record the modifications and updates of method.

SOP9 and Work Instruction 1 (WI1) provide general guidance on systematic selection of the adequate instrumental technique, extraction method, and spiking compounds for the Positive Control for each sample and on who makes the selections.

4.2. Major Practices

4.2.1. Initial Demonstration of Competency

Prior to analyzing the samples using the procedure described in section 3, the following exercise was performed by each analyst as Initial Demonstration of Competency (initial DOC). An analyte was selected from each of six major pesticide groups: chlorophenoxy herbicides, base-neutral herbicides, sulfonylurea herbicides, imidazolinone herbicides, fungicides, and insecticides. They were spiked on LCSs (one or two analytes per LCS) at levels often found in pesticide misuse samples. The spiked LCSs were given to each analyst to analyze, following the procedure described in section 3. The results were then graded against pre-established criteria and the grades were filed in each analyst's competency record folder.

The initial DOC was part of the demonstration of MDA lab's technical competence and depth of experience that support the granting of the flexible scope.

4.2.2. Proficiency Testing Plan

The MDA lab annually participates in the Pesticide Residue Check Sample Program provided by the Wisconsin Department of Agriculture, Trade and Consumer Protection, Bureau of Laboratory Services.

The MDA lab has been successfully completing the proficiency tests for vegetation matrix (corn matrix) using the procedure presented in section 3 since November 2014.

4.2.3. Method Development Record

Any method development activities carried out following the SOP8 are documented and filed in method development folders assigned to each analyte and are available for review at any time. They are also available to the analysts as reference for future method development activities.

5. Results and Discussion

The ISO17025 accreditation was granted to the procedure described in section 3 in May 2015 by A2LA under the flexible scope policy. With the presented procedure analytical methods used are validated for specific matrix/analyte combination for each plant matrix (each sample). The procedure provides the MDA lab with the fast validation and the flexibility to analyze samples with any matrix/analyte combination and allows the MDA lab to respond to pesticide misuse cases in timely manner.

5.1. Analysis Time

To help provide satisfactory sample turnaround time to the customer using the procedure the MDA lab simplified extraction methods. When applicable, extractions that involve time consuming and/or labor intensive steps such as separatory funnel were replaced with QuEChERS [7] based multi-residue extractions.

However, even with the simplified extraction, the simultaneous validation inevitably increases analysis time. As it is presented in Table 1 the number of samples to be extracted and instrument time increase by factor of 3 per sample using the accredited procedure. In the first year of implementation of the procedure (Y2015), analysts spent roughly twice as long per batch as the procedure without accreditation.

The longer sample turnaround time was predicted and was communicated to the customer at the beginning of Y2015. The MDA lab initiated close communications with the customer on sample prioritization and status updates in order to help compensate for the longer analysis time.

5.2. Reported Limit of Detection

Under the old procedure at the MDA lab (Table 1 Without Accreditation), the detection limit in a vegetation sample for an analyte was estimated based on the instrumental minimum detectable amount (the lowest level of a calibration curve) of an instrumental sequence for an analytical batch. When a batch consisted of a few different plant types, the same detection limit for an analyte was assigned to all plant types that were in the batch.

Under the accredited procedure the detection limit of each analyte is determined per plant type (foliage part of plant). Table 3 lists LODs of fomesafen determined for each vegetation

Corn	Kale	Peas	Apple	Weeds
4.02	14.4	3.46	0.61	2.01

Unit: parts per billion

Table 3: Formesafen LOD for select plant types.

sample. No fomesafen was detected in each sample at the prescreening. Each sample was then spiked with fomesafen at an estimated lowest level that can be recovered from the plant type. Then, the spiked samples were applied to the entire analytical procedure. The LOD was confirmed for each sample when the spiked fomesafen was recovered from the matrix by the analysis.

The LODs in Table 3 are not established by a statistical measure. However, a significant difference observed among LODs, confirmed as above for each vegetation type, reassures the importance of validating analytical method for each plant type.

5.3. False Negatives/Positives Rate

The false negative/positive rate is an important factor for evaluating an analytical procedure, especially when the results are used for regulatory purposes.

With the presented procedure, an incurred residue is analyzed and its presence is confirmed at least four times: prescreen, sample as it is, MSA-1, and MSA-2. When results of the four analyses of a sample show any discrepancy in regard to the presence of an incurred residue (e.g. target analyte is present in *prescreen* but not detected in *sample as it is*) further analysis and/or investigation will be conducted on the sample. Therefore, the established procedure helps prevent the laboratory from reporting false negative/positives and increases confidence in reported results.

The MDA lab's customer appreciated the value of the accreditation and was satisfied with the laboratory's performance under the accredited procedure. The information presented in this manuscript may be utilized by other regulatory laboratories who face similar challenges gaining ISO 17025 accreditation in their laboratories. However, ISO 17025 does not specify how the standards should be implemented in a laboratory. The procedures presented in the manuscript is specific to the MDA lab and its customer's needs. The responsibility of fulfilling the standard requirements is left to individual laboratories.

6. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

7. Disclaimer

This publication has also been presented in the 53rd North American Chemical Residue Workshop on July 17th to 20th 2016 in St. Pete Beach, Florida, USA.

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9. Article Information

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