Quality Assurance/Quality Control in the HPLC Pesticides Laboratory

1 SCOPE AND APPLICATION

1.1 This SOP details the Quality Assurance (QA) and Quality Control (QC) procedures for the HPLC-Pesticides Work Group.

1.2 Quality Assurance consists of all of the practices undertaken in a laboratory to insure the data generated is as accurate and precise as possible. It includes not only quality control measures, but can be as specific as the cleaning of glassware and preparation of standards. This SOP will concentrate on Quality Control measurements that are used to measure and track the Quality Assurance in the HPLC Pesticides laboratory. It will touch briefly on some general guidelines for QA. Refer to the specific preparation or analysis SOPs for more specific details on QA.

1.3 Most of the QA/QC practices described are common throughout the Chemistry Section.

2 DEFINITIONS

2.1 The quality control measurements used in the HPLC lab are: Accuracy, Precision, Blank Evaluation and Detection Limits (method detection and practical quantitation limits-MDL/PQL). These are briefly defined as follows:

2.2 Accuracy is the term, which describes the degree of deviation (bias) between a known amount of analyte added to a sample and the actual recovery amount of the analyte. It is expressed as the percent recovery.

2.3 Precision is the term, which describes the degree of replication between duplicate samples. It is calculated and expressed as the Relative Percent Difference (RPD) between duplicate sample results.

2.4 Blank Evaluation is used to assess the degree of contamination occurring from the sample preparation and analysis steps.

2.5 Detection limits (MDL/PQL) are established during the validation of a method. These limits define the sensitivity of a method. Because "zero" has no meaning when an analyte is not detected, a method analyte must have a limit set which defines the lowest concentration that can be reliably seen when it is detected.

2.5.1 Method Detection Limit (MDL): The minimum concentration of an analyte that can be identified measured and reported with 99 % confidence that the analyte concentration is greater than zero.

2.5.2 Practical Quantitation Limit (PQL) - Usually set at 3-5 times the MDL, it is a more reliable quantitation limit. Although the MDL can be detected at a 99 % confidence, the PQL is usually the point where the analyte can be quantitated more accurately.

3 IN-LAB QUALITY CONTROL SAMPLES

3.1 For every batch of 20 samples or less, the following quality control samples are prepared and analyzed:

3.2 One Laboratory Reagent Blank (used to evaluate contamination in the laboratory)

3.2.1 This is a reagent blank that is treated exactly as a sample, including exposure to all glassware, equipment, solvents and reagents used with other samples. The lab blank is used to determine if the method analytes or other interferences are present in the

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laboratory environment, reagents or equipment.

3.2.2 If contaminants are present in levels that result in interference with the determination of any analyte, detection limits MUST be elevated accordingly! If a lab blank is found to be contaminated, the source of contamination must be found and eliminated.

3.3 One Laboratory Fortified Blank (LFB)

3.3.1 A LFB is a reagent blank that is fortified at sample extraction with a known amount of the analyte(s) of interest and with any surrogates used.

3.3.2 A LFB may be prepared singly or in duplicate. It is used to assess the method accuracy (i.e., analyte % recovery) without the effects that a true sample matrix may contribute. If prepared in duplicate, it is also used to assess method precision (as Relative Percent Difference).

3.4 Matrix Spike Samples (for assessing method accuracy and if performed in duplicate, for assessing precision on "real" samples, in the presence of matrix effects.)

3.4.1 Sample matrix spikes are samples that are fortified at sample extraction with a known amount of an analyte(s) of interest.

3.4.2 Every attempt is made to prepare sample spikes in duplicate, in order to assess the true method precision in the presence of matrix effects. Spike recoveries (accuracy) are indicators of sample matrix interference, loss of analyte and contamination. Historical percent recoveries are used to calculate method bias and the confidence range of the bias. If insufficient sample is available to prepare matrix spikes in duplicate, LFB's are prepared in duplicate to substitute.

3.5 Other Quality Control measures that may be used are:

3.5.1 Instrument Blank (a solvent injection on the HPLC, to assess presence of possible instrument contaminants)

3.5.2 Internal Standard - a pure analyte(s) of known amount that is added to the final sample extract prior to HPLC analysis. It is used to measure the relative response of other analytes and surrogates and is useful in correcting for injection variations. It is also used as a retention time reference peak, to correct for retention time drift during an analysis sequence. The internal standard must be an analyte that is not expected to be found in the sample.

3.5.3 Surrogate - a pure analyte(s) that is added to all samples, blanks and spikes at sample extraction. Surrogates should be similar in behavior to the method analytes, but SHOULD NOT be expected to appear in the sample. Surrogate recoveries give an indication of method accuracy and are a good check for gross inaccuracy (i.e., lost sample, incorrect volumes, concentration problems, etc.) in all of the samples.

4 EXTERNAL QC SAMPLES

There are field QC samples that are assessed along with the samples. These are:

4.1 Field Duplicate Samples - two separate samples collected at the same time and from the same site under identical circumstances and treated exactly the same through all field and lab procedures. Duplicate analysis gives a measure of the precision associated with the sample collection, preservation and storage, as well as with the lab procedures

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4.2 Field Blank - reagent water added to a sample bottle at the time of sample collection. It is treated exactly as a sample, including exposure to the sampling site conditions, storage, preservation and all lab procedures. It is used to determine if method analytes and/or other interferences are present in the field environment.

4.3 Equipment Blank - After sampling, the equipment (baler, etc.) is rinsed with reagent water and collected as a sample. This blank is evaluated to insure that the field equipment did not contaminate the samples.

4.4 Field Spikes - These can be matrix or reagent water fortified samples that are used to assess the stability and method performance of an analyte(s). These may be "blind" spikes, which are unlabelled as spikes and are therefore unknown to the lab personnel. This is an excellent way to determine true method performance.

5 QC CALCULATIONS/REPORTING CONVENTIONS

5.1 Accuracy (expressed as % Recovery) of LFBs and matrix spikes is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Sample Concentration}}{\text{True Value}} \times 100\ \%$$

5.2 Precision (as Relative Percent Difference-RPD of the % recovery of duplicate LFBs and matrix spikes, or RPD of duplicate sample results) is calculated as follows:

$$\text{RPD} = \frac{|A - B|}{(A + B)} \times 200\%$$

Where

A = % recovery from spike 1 (or sample concentration from sample 1)

B = % recovery from spike 2 (or sample concentration from sample 2)

Note that this value is the Difference between the % recovery from sample A and B, divided by the Average of the 2 values.

5.3 Acceptance Limits are established for accuracy and precision by evaluating LFB/spike recoveries. These limits are used in assessing the acceptability of subsequent accuracy/precision of a spike and in determining if the value is a true and accurate reflection of the method performance. Limits are established for each matrix (i.e., sediment, water) and each analyte concentration level.

5.3.1 Accuracy Acceptance Limits consist of both Warning Limits and Control Limits:

5.3.1.1 Warning Limits are defined as the mean from a set of 20-25 data points (recovery values), +/- 2 Sigma. Sigma is the standard deviation of the n-1 population. This calculation will give an upper and a lower warning limit for the accuracy values. Warning Limits are used in evaluating data trends.

Upper Warning Limit (UWL) = Mean + 2 Sigma
Lower Warning Limit (LWL) = Mean - 2 Sigma

5.3.1.2 Control Limits are defined as the mean from a set of 20-25 data points (recovery values), +/- 3 Sigma. Sigma is the standard deviation of the n-1 population. This calculation will give an upper and a lower control limit for

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the accuracy values. Limits are used in accepting or rejecting recovery data and in determining whether a system is out of control. EPA defines an out-of control system as one in which there are seven successive data points on the same side of the mean value.

Upper Control Limit (UCL) = Mean + 3 sigma
Lower Control Limit (LCL) = Mean - 3 sigma

5.3.2 Precision Acceptance Limit

5.3.2.1 Warning Limit is defined as the mean from a set of 20-25 RPD values + 2 Sigma. This calculation will give an upper warning limit for the precision values. For precessions, only the upper limit is relevant.

5.3.2.2 Control Limits are defined as the mean from a set of 20-25 data points (RPD values), + 3 Sigma. This calculation will give an upper control limit for the precision values. For precision, only the upper limit is relevant. This limit is used in accepting or rejecting precision data and in determining whether a system is out of control. EPA defines an out-of control system as one in which there are seven successive data points on the same side of the mean value.

Upper Control Limit (UCL) = Mean + 3 sigma

5.3.3 The accuracy and precision limits are calculated from the latest 20-25 data points. These ranges are used in assessing the next 20 -25 data points as they in turn are generated. Once 20-25 new data points have been generated, new limits are calculated. If any data point is outside of the acceptance control limits, the batch of samples must be re-extracted provided that samples are not expired. If samples are expired, then any positive result for the unacceptable analyte result is qualified with "J". In addition, a comment is added to the SMP report detailing the outcome of earlier data.

5.3.4 All quality control limits, including LFB, sample matrix spikes, Surrogates and CCV, initially should be established based on either the criteria stated in the methods or initial validation data. When sufficient QC data (20 data points or more) become available, the quality control limits should be re-evaluated statistically and re-set when deemed scientifically appropriate.

5.3.5 If consistent deviations from QC limits, deemed to be caused by systematic errors of the procedure, are observed in LCS recoveries/precision, the analyst must proceed with 5.3.5.1 and 5.3.5.2.

5.3.5.1 Locate and correct the source of problem
5.3.5.2 Repeat the test for all parameters that failed to meet criteria

5.3.6 Repeated failure, however, will indicate a general problem with the measurement system. If this occurs, repeat 5.3.5.1 and 5.3.5.2 until the problem is solved.

5.3.7 In case the failures cannot be corrected, a new Initial Demonstration of Capability must be completed and new QC limits must be established within the acceptance criteria provided by the method.

5.3.8 For qualifying data in case of LCS failure due to a Systematic Error, see Laboratory SOP LB-027.

5.3.9 In QC Manager, if any QC failures in the batch are detected during calculations, do not

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add any comments to the effected samples. Other comments and qualifiers may be added as required.

5.3.10 In Data Review, if any QC failures are detected for any analyte during calculations, the effected analyte in each sample is automatically qualified with a comment ("QC failure(s) observed"). This comment is coded in Data Review calculations so that it is only appended once in the case of multiple QC failures.

5.3.11 Following DEP SOP LB-027, the effected data must be qualified with a proper comment in the final report explaining any deviation from the routine limits whenever the spikes or surrogates and or CCVs are out of range. The following can be used as needed: One or more results were qualified due to non-systematic CCV failures (or matrix spike failures or LCS failures or lab QC failures).

5.3.12 Rules for J qualifying data: Failing LFBs (recoveries or RPDs): For all samples in the batch, the component with the failing QC is J'd and appended with a comment. Failing matrix spike recoveries or sample/matrix spike precision: The component with the failing QC for the spiked/duped sample only is J'd and appended with a comment.

5.3.13 General rules: J is added to all results regardless of qualifier code (i.e. UJ, IJ, YJ etc.). The one exception is data reported as O which should not be assigned the J code. Do not want to add a J code if it already exists, i.e. no JJ's.

5.3.14 Do not load precision if matrix spike recovery is none detect and or less than PQL, load matrix spikes recoveries and make comment.

5.3.15 If analyte in sample is higher than spike level, do not load matrix spike recoveries, make comment, do not qualify result and report RPD.

5.3.16 Check the method blank for any interfering contaminants. The MDL's may have to be elevated if the blank(s) indicate contamination.

5.3.17 LCS failures allowable by NELAC chapter 5, Appendix D, section D 1.1.2:

> 90 analytes in the spike – 5 analytes are allowed to fail
71 - 90 analytes in the spike – 4 analytes are allowed to fail
51 - 70 analytes in the spike – 3 analytes are allowed to fail
31 - 50 analytes in the spike – 2 analytes are allowed to fail
11 - 30 analytes in the spike – 1 analytes are allowed to fail
< 11 analytes– no analytes are allowed to fail

5.4 Rounding of Numbers

See SOP **LB-024** "Significant Figures: Policy of the DEP Chemistry Section" for details.

5.5 LIMS Reporting Codes/Conventions

5.5.1 Sample results that are below the PQL's and/or MDL's have to be reported with a remark code (qualifier) in LIMS. Samples that are analyzed in duplicate (in-lab duplicates, NOT field duplicates) must be reported with a qualifier. Samples that are analyzed after expiration must be reported with a qualifier.

5.5.2 For sample results that are below the MDL report the MDL followed by the letter U.

5.5.3 For sample results that are below the PQL but above the MDL, report the sample result followed by the letter I.

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5.5.4 Any sample extracted and analyzed in duplicate (in-lab duplicates, NOT field duplicates) report the average of the results followed by the letter A.

5.5.5 For samples that are analyzed after expiration report the result followed by the letter Q.

5.5.6 Some conditions may require two or more qualifiers. Report only one qualifier. Use the list below to assign priorities (#1 has highest):

1. U or I
2. Q
3. A

5.5.7 Request approval by the supervisor before using any qualifiers that are not listed above.

5.5.8 Report all water results in µg/L, all sediment and waste samples in µg/kg.

6 MDL/PQL DETERMINATION/METHOD VALIDATION

6.1 MDL and PQL values are initially determined during validation of a new method.

6.2 MDL: Estimate the MDL by analyzing a standard on the appropriate instrument and examining the chromatographic peak. The peak should at least have signal–to-noise ratio (S/N) of five (5).

6.3 After obtaining the estimated MDL, seven (7) replicates lab-fortified blanks are prepared at 2-5 times the MDL level. These LFBs are then analyzed. The relative standard deviation should be acceptable, based on EPA method or other references results (usually not > 30%). If this is achieved, the MDL of that analyte is determined following EPA 40 CFR Part 136, Appendix B and Revision 1.11. If the criterion is not achieved, 7 replicates LFB's at higher concentration are prepared and analyzed until the criterion is achieved.

6.4 PQL: The PQL is usually set at 2-5 times the MDL level.

6.5 Determined MDL and PQL are used when reporting data. However, these MDL/PQL values may need to be adjusted when analyzing real samples, to account for differences in matrix interference, sample wet/dry weight and amount of sample analyzed.

7 NELAP

7.1 To keep valid NELAP certification current for all the analytes in each test method for each matrix, the technical personnel need to complete all the requirements every year and on-going demonstration of capabilities. Certification is required if any changes to the SOPs, any changes in the preparation, analysis and MDL changes. For successful certification each person need to complete 4 QC samples or one Performance Evaluation sample every year.

7.2 Once a year, run each test method for routine samples and participate if available in PT samples and/or if no routine and PT samples are available then do 4 QC samples. Spike each analyte for each matrix.

7.3 For multi-component analytes rotate spike and prepare spike rotation schedule. For all the HPLC analysis, for each method all the analytes are spiked and analyzed.

7.4 In each run sequence, check instrument detection limits by running close to MDL standards.

7.5 For each Method and Analyte, run once a year MDL Check Spike by extracting and analyzing one MDL Check Spike (3 to 5 times of MDL). The following Table lists the MDL Check Spike concentration for all the HPLC methods.

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Table: LOD Check – Amount of Spike Standard Required

Method	Reported MDL in LIMS In PPB	On Column Conc. (µg/L) Required for MDL	Lowest ICAL Standard (µg/L) Used for Analysis	LOD Check Conc. on Column (µg/L)	Routine LFB's (µg/L)	LOD Check Spike Amount (mL)	LOD Spike Conc.
W-AHERB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-AHERB-AA	6.5	20	0.5	100	100	0.1	10 ppm
W-CARB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-CARB-AA	1.0	1	0.50	5	25	0.05	1 ppm
W-GLYPST	10	10	10	50	100	0.5	1 ppm
W-PDQUAT	1.0	50	100	200	1000	0.20	5 ppm
W-MCYST-AA	1	1	1	5	10	0.5	200 ppb
W-SUC-AA-R	0.010	10	10	50	250	0.05	500 ppb
W-UHERB-AA	0.0020	0.5	0.5	2.5	25	0.025	0.10 ppm
S-UHERB-AA	1.0	1	0.5	5	25	0.05	1 ppm

8 QUALITY ASSURANCE

8.1 The following are general guidelines to use in assuring that good and complete data be reported. This is not a complete list of guidelines!

8.2 All data and quality control data are reviewed by a supervisor before being accepted as final.

8.3 All maintenance or analytical problems are reported to a supervisor.

8.4 Field sampling problems such as contamination, labeling, and holding times are reported to a supervisor and noted with the results.

8.5 Document quality control from all analyses sets in an established QC notebook.

8.6 Document QC deviations in the final SMP report by adding appropriate comments and by flagging any affected results with the proper code.

8.7 Method interferences may be caused by contaminants in reagents, glassware, instrumentation, and highly contaminated samples. All of these contaminants must be routinely monitored by daily analysis of laboratory water blanks. To avoid method interferences, take the following general precautions:

8.7.1 Solvents

8.7.1.1 Check each batch of solvents by concentrating and analyzing for purity before use.

8.7.1.2 Use only the specified grade of solvents at all times. If interferences form solvents are suspected, analyze a concentrated aliquot of the solvent to check this. Remove any contaminated solvent from production IMMEDIATELY. Label solvents as contaminated.

8.7.1.3 Never use solvents from unlabelled bottles.

8.7.1.4 Never pipet solvents directly from bulk solvent. Always take the required volume of solvent into a labeled beaker and pipet from this. Dispose of the

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unused portion.

8.7.2 Reagents

8.7.2.1 Use the purity of reagents recommended by the appropriate SOP.

8.7.2.2 Store all the reagents appropriately in a clean environment, and with a proper label, which includes the date and initials of the person who prepared the reagent.

8.7.3 Use scrupulously cleaned glassware. See SOP for further details

8.8 BE SENSITIVE TO CONTAMINATION AT ALL TIMES!!! If a highly contaminated sample has been analyzed with other non-contaminated samples, be sure that any positives reported in the other samples are true! Positive results seen in other samples may be carry-over from this highly contaminated sample.

8.9 Any sample determined to be positive for an analyte must be confirmed using with a second HPLC/GC column analysis or by a spectroscopic (mass spec/ UV photodiode array) technique!

9 REPORTING, DOCUMENTATION AND DATA ARCHIVAL

9.1 When analysis is complete, and then downloads the final sample results into the Laboratory Information Management System (LIMS) using QC Manager or Target. If a sample has been diluted or has a dry weight less than 100 %, then make sure that the information was entered properly into the QC Manager or TARGET.

9.2 Add the appropriate qualifiers and comments to the sample data in the QC Manager after performing a complete review of the sample data and QC data. See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

9.3 Definitions of the available qualifying codes are listed in DEP SOP LB-027

9.4 All quantitation reports for samples and QC should be placed in a results folder labeled with the job number, the sample numbers, and the type of analysis.

9.5 Retrieve the field folders (containing the field sheets) and check the data against the field sheets to see if it makes sense (i.e., does the equipment blank contain several hits while an effluent sample does not?) If anything looks suspicious, further research is necessary before posting results.

9.6 Enter the results for each sample in the analysis results form. Print out QC results from the QC Manager. Include a copy of dilutions page if any. Also include a print out of sample positives from the data review and file it in the data folder.

9.7 Submit the result folder to the section supervisor. Make a copy of results page, positive detection page and run sequence, and file it in the results notebook. The supervisor will review results and then authorize the sample. The supervisor will then file the folders in the lab. The supervisor will notify the Environmental Manager of the organic chemistry section to authorize the job.

9.8 Original analysis data collected in LC-MS Target is archived in to TLHLAB3/LC/LCMSBulkArchive and in Target data is archived by computer group. All of the QA/QC data and analysis results are archived in the proper file kept in HPLC Lab by chemist who performed the analysis.

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10 REFERENCES

10.1 Meier, P.C. & R. Zund, "Statistical Methods in Analytical Chemistry", Vol. 123 of Chemical Analysis: A Series of Monographs on Analytical Chemistry and Its Applications, John Wily & Sons, Inc., New York, 1993.

10.2 "EPA: Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act", 40 CFR, Part 136, Oct. 1984.

10.3 "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Environmental Protection Agency, EPA-600/4-79-019, March 1979.

10.4 EPA SW-846, including all updates.

10.5 DEP SOP LB-027. *Standard Operating Procedure for Reporting Qualified Data.*

10.6 DEP SOP LB-024. *Significant Figures: Policy of the DEP Chemistry Section* for details.

Appendix of Changes

08/15/2012 Minor changes to spike standards addition.

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