وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

ANALYTICAL QUALITY CONTROL (AQC)

FOR PESTICIDE RESIDUES ANALYSIS IN FOOD

Dr.Gouda Abdullah Ramadan

Consultant, Food Safety Laboratories
Food safety Department
Doha-Qatar
ggoadah@moph.gov.qa

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Purpose of Presentation

The guidance in this PPT is intended for laboratories involved in the official control of pesticide residues in food in accordance with *SANTE guidelines, 11312/2021-v2*

To describes the Analytical Quality Control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure.

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

The key objectives

To provide a harmonized, cost-effective quality assurance and quality control system.

To ensure the quality and comparability of analytical results.

To ensure that acceptable accuracy is achieved.

To ensure that false positives or false negatives are avoided.

To support compliance with and specific implementation of ISO/IEC 17025 (Accreditation Standard)

Experimental Method Workflow

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Receiving of samples

Sample preparation

Prepare Mobile phase and LC Column

Spike preparation

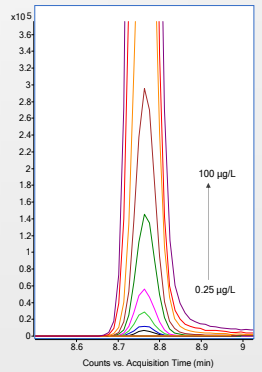
Prepare Sequence

Data analysis

Check acceptance criteria & Recovery

Assign **MRL**

Result Reporting in **LIMS**



Sample Preparation Workflow

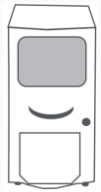
وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Sample Extraction

Sample
(10.0 ± 0.1g)

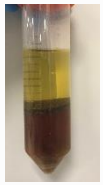
- Add 10ml of ACN
- Shake 1 min

QuEChERS
extraction
Mixture



Mechanical
Shaker or
Handshake 2 min

Centrifuge
10min



QuEChERS Extraction

Sample Cleanup

Transfer
supernatant

D-SPE tube

Centrifuge
5 min

Sample injection

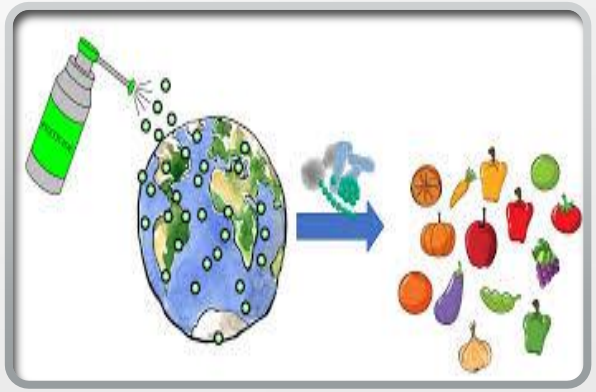
LC-MS/MS
GC-MS/MS

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Calibration for Quantification

General requirements

- The lowest calibration level (LCL) must be *equal to, or lower than*, the calibration level corresponding to the RL. The RL must *not be lower than* the LOQ.
- Multi-level calibration (three to five or more concentrations) is preferred. An appropriate calibration function must be used (e.g. linear, with or without weighing).
- The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region *should not be more than ±20 %.*
- Analytes for calibration : All targeted analytes must be injected in every batch of samples, at least at the level corresponding to the RL. Sufficient response at this level is required and should be checked to avoid false negatives.



RL= Reporting Limit

Matrix-matched Calibration & Matrix Effects Studies

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

- Matrix effects are known to occur frequently in both GC and LC methods and should be assessed at the initial method validation stage.
- Matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same type as the sample, should be used for calibration.
- An alternative practical approach to compensate for matrix effects in MS/MS-analysis is use of Standard addition of at least two known quantities of analyte to aliquots of the sample extract, prior to injection (extract standard addition).
- In this case adjustment is only for matrix effects for individual commodities. (This approach is recommended in multidisciplinary government regulatory labs)

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Matrix Effect Evaluation – Ensuring Accurate Results

- Pesticide residues analysis is challenging due to Matrix effect (ME) behaving in terms of ion suppression or enhancement of the MS detection system response.

$$\text{ME Factor} = \frac{Target\ Response\ in\ solvent}{Target\ Response\ in\ matrix}$$

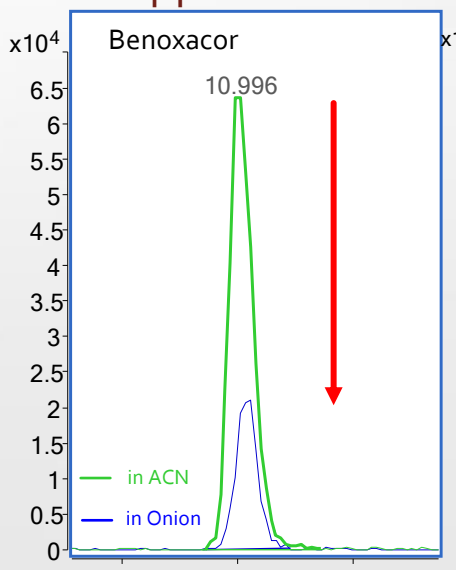
The analyte Reporting concentration in sample(Cs- µg/ml) is calculated as follows..

Cs = Ci * ME Factor

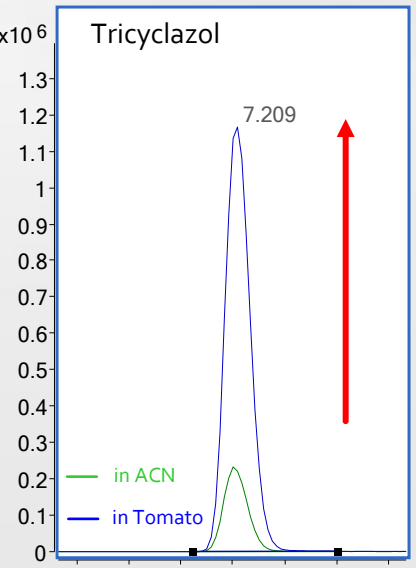
Ci= Found concentration in sample(µg/ml **)**

ME Factor= Matrix Effect Factor

suppression



Enhancement



وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

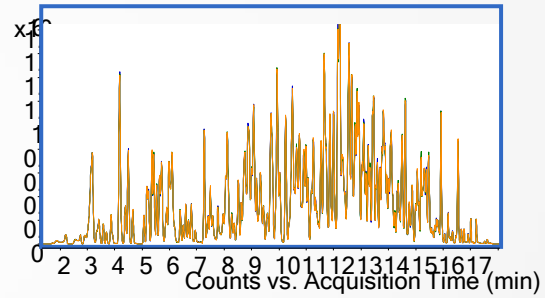
Sequence table format for LC-MS/MS and GC-MS/MS

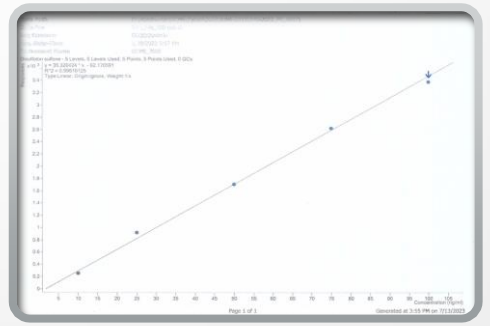
S.N	Type	
1	Reagent Blank	→ To check interference
2	Multilevel Calibration(5 or more points)-**Weekly**	→ To check linearity(**more than 0.99**)
3	Verification (Mid point calibration)-**Daily**	→ To verify accuracy**(±20%)**
4	QC samples(CRM, Spiking and related **ME**)-**Daily**	→ To Check recovery (**70 to 120%)**
5	Standard in matrix (matrix interference)-**Daily**	→ To evaluate **Matrix Effect**
6	**Routine samples**	→ To find unknown samples concentration.
7	**Bracketing standard** every 20 injections and at end of the sequence	→ To verify the efficiency of the calibration, detect any deviation from the specified criterion **(±20%)**
8	Wash/ Conditioning	

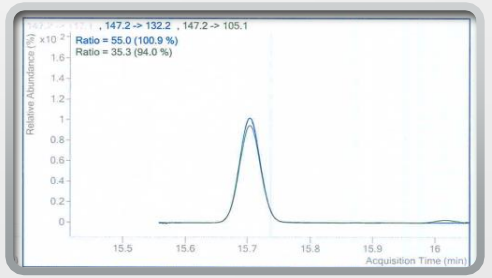
- **Note: It's not permitted to inject all standard matrix weekly once**

Data processing

- Chromatograms must be examined by the analyst and baseline fit checked and adjusted, if necessary.
- Where interfering or tailing peaks are present, a consistent approach must be adopted for the positioning of the baseline.
- Where practicable, recoveries of all analytes in the scope should be measured within each batch of analyses.
- If this requires a disproportionately large number of recovery determinations, the number of analytes may be reduced. However, it should be following the minimum number specified in Table 1. This means, that at least 10 % of the analytes.
- In the Food Safety Laboratory, the recovery of pesticides residues that are determined in routine samples is studied with each batch.







Data processing

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Table 1. Minimum frequency of recovery checks (quantitative method performance verification)

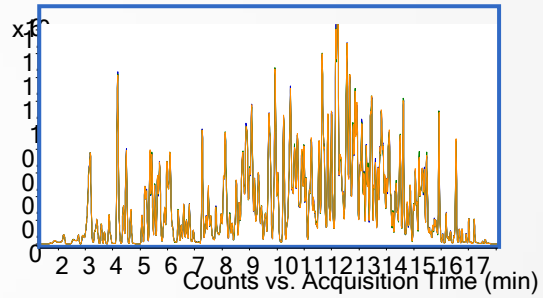
Test	Analytes for recovery check (minimum)	All other analytes
Number of analytes	At least 10 % of the scope per detection system covering all critical aspects of the method	Within a rolling program to include all other analytes as well as representative commodities from different commodity groups
Minimum frequency of recovery checks	Every batch	At least every 12 months, preferably every 6 month
Level	RL	RL

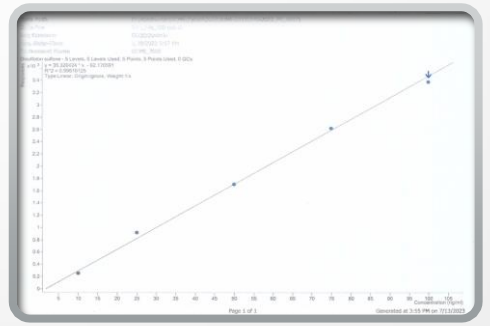
Data processing

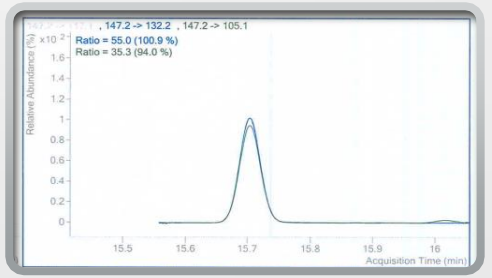
وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Acceptance criteria:

- In calibration plotted curve for each pesticide shall be **linear** with correlation coefficient(R2) **more than 0.99**
- Recovery of verification standard & Bracketing std not exceed ±20%.
- In multi level calibration minimum 3-points required for Quantification.
- **Retention time (Rt)** of the suspected analyte compared with retention time of the standards
- The peak shape of the analyte and Relative abundance of the recorded masses (in general 2 MRM transitions are required in MS/MS).
- Spiking recovery should meet the criteria(CRM or Quality Control)







Confirmation of results

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

- If the initial analysis does not provide unambiguous identification or does not meet the requirements for quantitative analysis, a confirmatory analysis is required.
- This may involve Reanalysis of the extract or the sample.
- When a MRL is numerically exceeded, a confirmatory analysis of another analytical test portion is required in case of potential legal actions.
- For unusual pesticide/matrix combinations, a confirmatory analysis is also recommended.
- The use of different determination techniques and/or confirmation of qualitative and/or quantitative results by an independent expert laboratory will provide further supporting evidence.

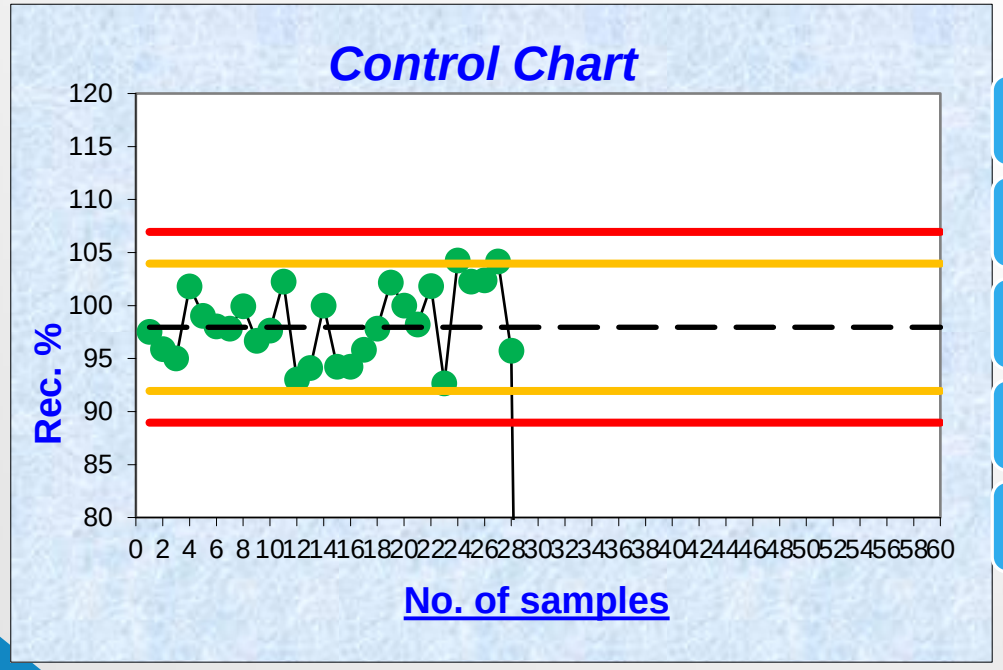
Reanalysis

Confirmatory analysis

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Quality control chart

Quality control chart of analytical results is an integral part of work to discover trends in **Reliability and precision of analytical results**.



The key objectives are:

- To ensure the quality and comparability of analytical results
- To ensure that acceptable accuracy is achieved
- To avoid that false positives or false negatives
- To compliance with ISO/IEC 17025 (accreditation standard)

- **Note: Ensure Quality control chart before reporting of the results.**

Reporting results

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

- The results from the individual analytes measured must be reported and their concentrations expressed in mg/kg.
- For quantitative methods, residues of individual analytes below the RL must be reported as <RL mg/kg or as <LOQ mg/kg .
- Where the extract of the same analytical test portion is analyzed by two techniques, preference should be given to the result which is considered to be the most accurate.
- Where two results are obtained by different equally accurate techniques or by replicate measurement(s) of an analytical test portion of the homogenized sample using the same technique, the mean of the result may be reported.
- In this case there are two replicates the relative difference of the individual results should not exceed 30 % of the mean.

Procedure for Selection of MRL

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Strategy for judging the results of pesticide residue samples to verify the maximum residue limit as follows the order respectively :

- **GSO** sets Maximum limits of pesticide residues in agricultural and food products GSO 382:2021
- **Codex** Alimentarius Commission by Food and Agriculture Organization (FAO) and World Health Organization(WHO) has set MRLs database for Pesticide residues..
- **European** Food Safety Authority (EFSA) has set EU MRLs database for pesticide residues.
- **USA**, Environmental Protection Agency(EPA) sets MRLs for Pesticide residues.

هيئة التقييس الخليجية
GCC Standardization Organization

Codex Alimentarius
International Food Standards

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Procedure for Selection of MRL

- Considering the following previous order, Search the name of the pesticide with the name of the food commodity/Category.
- If there are no individual limits for the compound with the specific food commodity ,then use approved Commodity sub-groups in the Codex and GSO standards.
- If limits are not available in GSO and Codex, European Union standard is resorted to.
- ***Refer: Codex Commodity Categorization***

Procedure for Assigning of MRL

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

GSO

- Compound Vs Specific Commodity (*If MRL not found, then search)*
- Compound Vs Group commodity

If not found GSO, Search in Codex

Codex

- Compound Vs Specific Commodity (*If MRL not found, then search)*
- Compound Vs Group commodity

If not found Codex, Search in EU database

EU

- Compound Vs Specific Commodity (*If MRL not found, then search)*
- Compound Vs Group commodity

Example for Assigning of MRL

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

GSO

- **Compound Vs Specific Commodity**
  - X pesticide Vs Mango ----------→ MRL Not found
  - *(Search again)*
- **Compound Vs Group commodity**
  - X pesticide Vs Assorted tropical fruits(Inedible peel)---→

Found MRL **Z** mg/kg

هيئة التقييس الخليجية
GCC Standardization Organization

Codex Alimentarius
International Food Standards

Categorization of Vegetables

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Codex Alimentarius
International Food Standards

Fruiting vegetables (cucurbits)	Fruiting vegetables (Other than cucurbits)	Bulb vegetables	Brassica vegetables	Leafy vegetables	Legume vegetables	Root and Tuber Vegetables	Stalk and Stem Vegetables
Cucumber	Peppers/Chili pepper	Garlic	Cabbage	Grape leaves	Beans	Radish	Celery
Bottle gourd/Bitter gourd/ sponge, wax gourd	Eggplant	Leek	Broccoli	Chard/A	Peas	Turnip	Artichoke
Melon	Okra/Ladyfinger	Fennel bulb	Brussels sprouts	Water cress		Sweet potato, Potato	Asparagus
Zucchini	Tomato	Spring onion	Cauliflower	Lettuce		Beetroot / Carrot	
Courgette	Sweet corn	Onion		Mallow		Yam	
Squash	Mushroom			Purslane			
Pumpkin				Radish leaves			
Gerkin/ Tindly				Spinach			

Categorization of Fruits

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Codex Alimentarius
International Food Standards

Berries and Other small fruits	Citrus fruits	Pome fruits	Stone fruits	Tropical Fruits (Inedible peel)	Tropical Fruits (Edible peel)
Blueberries	Lemon/lime	Apple	Cherries	Custard apple	Date
Strawberry	Mandarin	Pear	Apricot	Avocado	Fig
Gooseberries	Orange	Persimmon (Kaka)	Plums	Banana	Jujube
Grapes	Grapefruit	Loquat	Nectarine	Jackfruit	Olives
Raspberries	Clementine	Quince	Peach	Kiwi	Guava
Currants	Pomelo	Medlars	Prunes	Longan/Litchi	Kaki fruit
Elderberries	Tangerine			Mango	Cashew apple
	Ugli			Papaya	Persimmon
				Pomegranate	
				Pineapple	

Codex Alimentarius
International Food Standards

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Categorization of Herbs & Spices

Herbs	Spices	Dried fruits
Basil leaves	Basil seed	Dried grape (Raisins, sultanas, Currants)
Curry leaves	Fennel seed	Dates
Dill leaves	Anise seed	Figs
Parsley leaves	Cardamom	Sultanas
Rosemary	Cinnamon	
Coriander leaves	Cloves	
Mints	Coriander seed	
Thyme	Cumin seed	
Celery leaves	Pepper	

وزارة الصحـــة العامـــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Questions and clarifications

وزارة الصحـــــة العامـــــة
Ministry of Public Health
State of Qatar • دولـــة قطـــر

Thank You All

Presented by

Dr. Gouda Ramadan