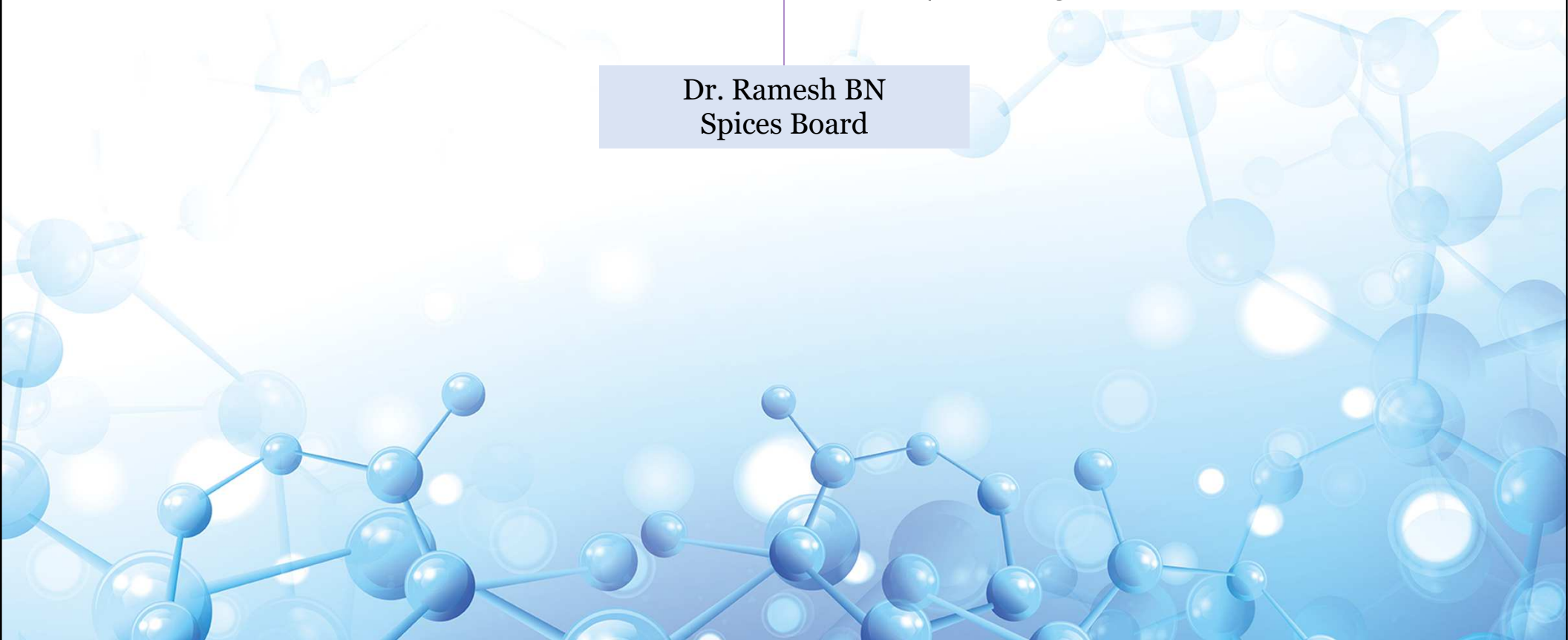


QEL, Spices Board

*In-house Training – 1:
Analysis of Pesticide Residues*

Lecture - 3
**Introduction Modern Pesticide
Residue Analysis**
17-Jul-23

Dr. Ramesh BN
Spices Board



Evolution of Pesticides

- Chemicals have been used throughout history to ensure **protection of crops** against pests
- Pesticides **evolved** along a path of increasing sophistication
 - **Historic** pesticides were elements like sulphur, arsenic etc. used in different forms
 - **Classical** pesticides (early 20th century) had low toxicity but high environment persistence
 - **Modern** pesticides have high toxicity, but low environmental persistence
 - **Emerging** pesticides have high toxicity, low environmental persistence and have higher specificity towards target pests

Pesticide residues

Ideally, a pesticide would be:

- **Toxic** to target organisms
- **Safe** towards non-target organisms and the environment

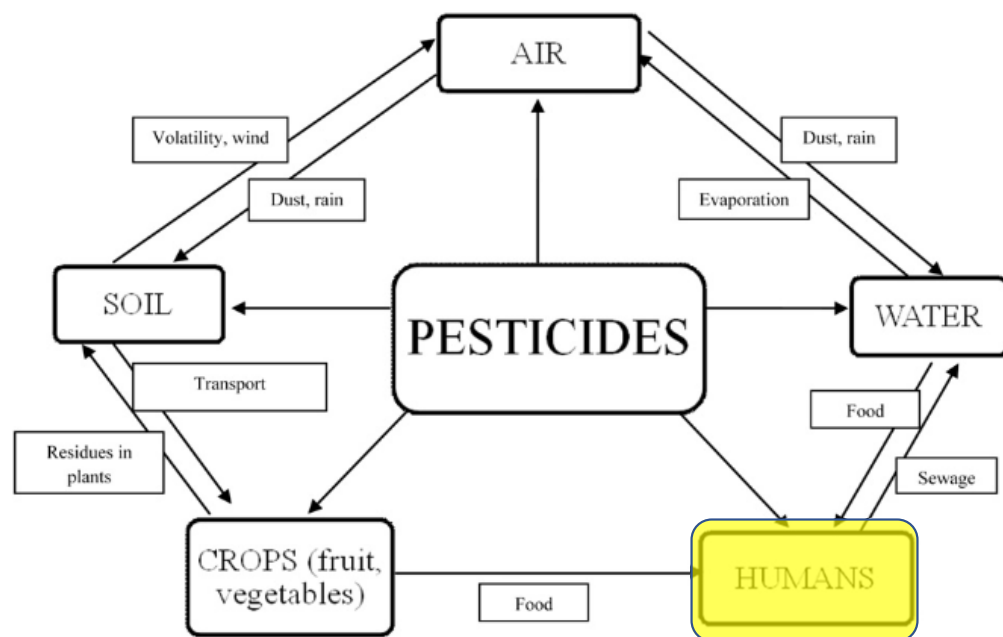
Issues:

- Target organisms develop resistance, and GAP are not always followed: farmers might use pesticides indiscriminately
- Several classes of pesticides of varying ranges of toxicity – not always harmless to nontarget organisms and environment



Pesticide residues in food

Environmental fate of residues



Properties and determination of pesticides in fruits and vegetables, Fenik *et al.*, *Trends in Analytical Chemistry*, Vol. 30, No. 6, 2011

Pesticides can permeate into every aspect of the environment, and **circulate**

Indiscriminate use is extremely hazardous

- + Increased agriculture productivity
 - + Reduce losses during food storage & transport
 - + Prevents food-borne diseases
 - + Increase durability of industrial products
-
- Affects non-target organisms and environment
 - Pests develop resistance
 - Threat to human life
 - Disrupts international food trade
- } **Testing for pesticide residues**

Maximum Residue Limits

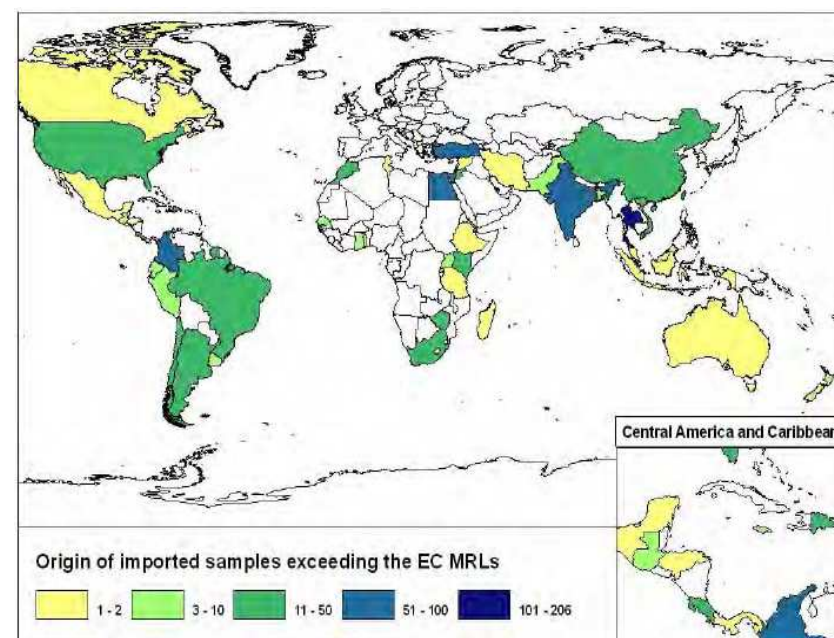


Testing is required to ascertain that food complies with MRL regulations

- Maximum allowed residue level in food, prescribed by regulatory agencies in different countries
- Designates the concentration of a residue in food, above which the food becomes unsafe for human consumption
- Typically expressed in mg/kg
- Pesticides will have different MRLs in different foods
- MRLs might vary from country to country
- In India, FSSAI is the regulatory authority issuing MRLs

Pesticide residues in trade

- In Europe, Indian food exports figure significantly in **MRL exceedance**
- In spices export also, MRL exceedance is a major concern, **second only to biological hazards** (Salmonella)
- Testing and clearing spices with respect to MRL compliance is important to maintain India's position in global trade



Data published by EU

TESTING FOR

PESTICIDE RESIDUES

Sampling for PRA



Process of drawing a [representative laboratory sample](#) from a large sample lot.

Complex topic, depends on the [product](#) being sampled...

Important considerations:

- Sampling frequency and sample weight
- Method of sampling - Different for different materials
 - Packed food - \sqrt{n} method
 - Soil – ‘W’ pattern
 - Water – stagnant? Flowing?
- Ensure sample consistency and integrity until it reaches the laboratory

Study [available literature](#)

Classes of pesticides

	Class of pesticide	Examples	Mode of functioning	
1	Organochlorines	BHC, DDT	Affects chloride ion transport at nerve centres	Classic
2	Organophosphates	Ethion, chlorpyrifos	Acetylcholinesterase inhibition	
3	Carbamates	Carbaryl, aldicarb	Acetylcholinesterase inhibition	
4	Pyrethroids	Allethrin, cypermethrin	Affect sodium channels in the axonal membranes	Modern
5	Neonicotinoids	Imidacloprid, acetamiprid	Binds to nicotinic acetylcholine receptors in cells	
6	Macrocyclic lactones	avermectins and milbemycins	Inhibition of chloride ion flow in cells	Emerging
7	Phenyl pyrazoles	Fipronil	Affects chloride ion transport at nerve centres	
8	Nereistoxin analogues	Cartap, thiosultap	Binds to nicotinic acetylcholine receptors in cells	
9	Diamides	Flubendiamide, chlorantraniprole	Releasing stored calcium from the sarcoendoplasmic reticulum	
10	Benzoylureas	ufenuron, triflumuron	Inhibition of biosynthesis of chitin	
11	Cyclic ketoenols	Spirodiclofen, spiromesifen	Acetyl-CoA-carboxylase inhibition	

Effectiveness
Specificity



Environmental
persistence

Multiclass, multiresidue methods

- Methods that can cover many classes of pesticides together
 - Ease of use
 - Broad applicability
- Issues:
 - Pesticides vary in chemical properties (solubility, polarity, stability...)
 - Covering a large range of pesticides in a single method: difficult
 - Classical multiresidue methods were limited in scope (extensive chemical procedures were needed in extraction stage)
- Modern methods:
 - Highly sensitive instrumentation
 - Extraction chemistry has become less complex

Classical and modern methods

- Classical methods (prior to 2003)
 - Required **large** sample **sizes** and solvent **volumes**
 - Multiple steps: labor intensive, time consuming
 - **Class-selective** methods
 - The **Mills** method (**USFDA-PAM**): Extraction in PE, partitioning in ACN, cleanup by florisil, concentration, reconstitution in multiple solvents, **GC** analysis
 - The **Luke** method (**AOAC**): Extraction in acetone, partitioning in PE and DCM, cleanup by florisil, concentration, reconstitution, **GC** analysis
- QuEChERS (from 2003)
 - **Low** sample sizes, solvent volumes
 - **Not labor intensive**, simple, quick and straightforward
 - Requires **GC-MS/MS** and **LC-MS/MS**

RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

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Public Health Institute, Environmental Protection Institute, Prvomajska 1, 2000 Maribor, Slovenia

FRANK J. SCHENCK

³U.S. Food and Drug Administration, Office of Regulatory Affairs, Southeastern Regional Laboratory, 60 Eighth St, Atlanta, GA 30309

A simple, fast, and inexpensive method for the determination of pesticide residues in fruits and vegetables is introduced. The procedure involves initial single-phase extraction of 10 g sample with 10 mL acetonitrile, followed by liquid-liquid partitioning formed by addition of 4 g anhydrous MgSO₄ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid-phase extraction (dispersive-SPE). In which 160 mg anhydrous MgSO₄ and 25 mg primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile extract. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, and sugars, to some extent from the food extracts. Gas chromatography/mass spectrometry (GC/MS) is then used for quantitative and confirmatory analysis of GC-amenable pesticides. Recoveries between 85 and 101% (mostly >95%) and repeatabilities typically <5% have been achieved for a wide range of fortified pesticides, including very polar and basic compounds such as methamidophos, acephate, omethoate, imazalil, and thiafendazole. Using this method, a single chemist can prepare a batch of 8 previously chopped samples in <30 min with approximately \$1 (U.S.) of materials per sample.

Pesticide residue analysis of food and environmental samples has been performed in numerous government and private laboratories throughout the world for approximately 40 years. However, the methods used for analysis of common pesticides are far from ideal. Some residue monitoring laboratories still use methods developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, extended analysis time and manual labor were the norm, and technology was less capable than today. Modern residue monitoring programs, however, are expected to be responsive to the latest developments in agriculture and new legislation. The introduction of new, more rapid, and effective analytical approaches, therefore, is essential for laboratories to improve overall analytical quality and laboratory efficiency.

Without question, the most efficient approach to pesticide analysis involves the use of multiclass, multiresidue methods (MRMs). The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills (1). At that time, nonpolar organochlorine insecticides (OCs) were the main focus for analysis. With the Mills method, OCs and other nonpolar pesticides were extracted from nonfat foods with acetonitrile (MeCN), which was then diluted with water, and the pesticides were partitioned into a nonpolar solvent (petroleum ether). As a consequence, relatively polar pesticides, such as certain organophosphorus insecticides (OPs), were partially lost during this step. The need to analyze more polar OPs and other pesticides in agriculture initiated the development of alternative procedures to determine compounds not extracted by the Mills method. These methods often simply modified the Mills procedure by using the initial MeCN extract but with different partitioning, cleanup, and determinative steps (2-4).

In the 1970s, new methods were developed to extend the analytical polarity range to cover OCs, OPs, and organonitrogen pesticides (ONs) in a single procedure (5, 6). These multiclass MRMs differed from the Mills approach in that acetone, rather than MeCN, was used for the initial extraction. However, the new methods still used nonpolar sol-



M Anastassiades



S J Lehotay

<https://www.quechers.eu/>

Home • Method • Literature • Links • Contact

Home of the **QuEChERS** Method

QuEChERS stands for

Quick Easy Cheap Effective Rugged Safe

and is the acronym for a highly beneficial analytical approach that vastly simplifies the analysis of multiple pesticide residues in fruit, vegetables, cereals and processed products thereof.

[Learn more](#)

Received September 4, 2002. Accepted by 25 October 25, 2002.

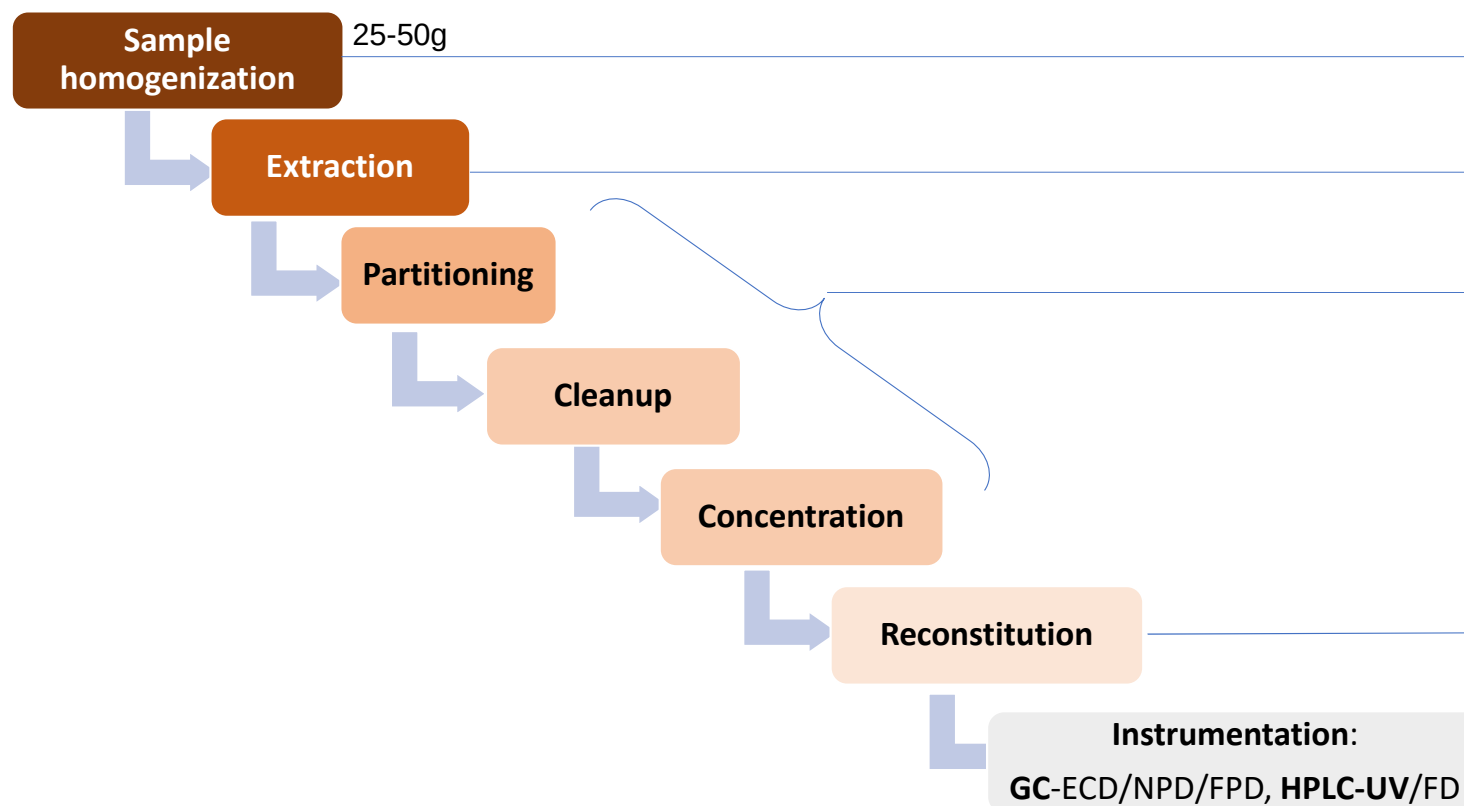
Current address: Chemisches und Veterinäruntersuchungsamt Stuttgart, Schöfendorferstrasse 3/2, 70376 Fellbach, Germany.

* Author to whom correspondence should be addressed; e-mail: anastassiades@arserrc.gov.

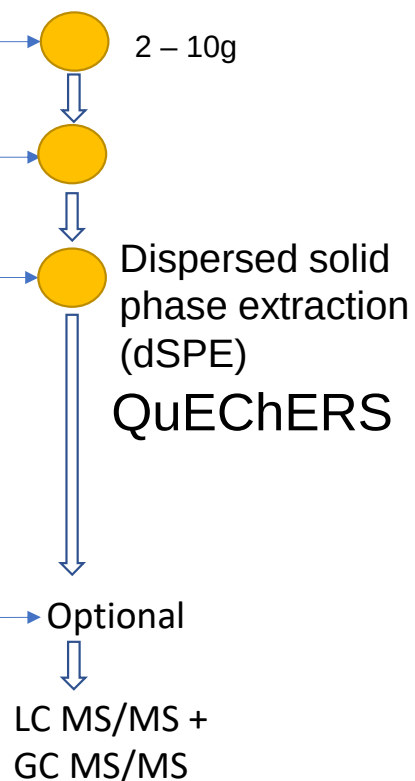
Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

Steps in a multiresidue method

Classical Multiresidue method



Modern Multiresidue Method



Sample preparation in the laboratory

- Ensure that the sample is thoroughly mixed before weighing out for analysis
- Grind the sample for further homogenization (not necessary for non-systemic pesticides)
- Ensure that no cross contamination occurs
- Ensure that no unintentional contamination occurs (e.g. low grade rubber in pipette bulbs, powdered latex gloves etc. can cause interference in mass spectrometer)
- Use MS grade solvents for analysis

QuEChERS (1)

Quick, Easy, Cheap, Effective, Rugged, Safe



quechers



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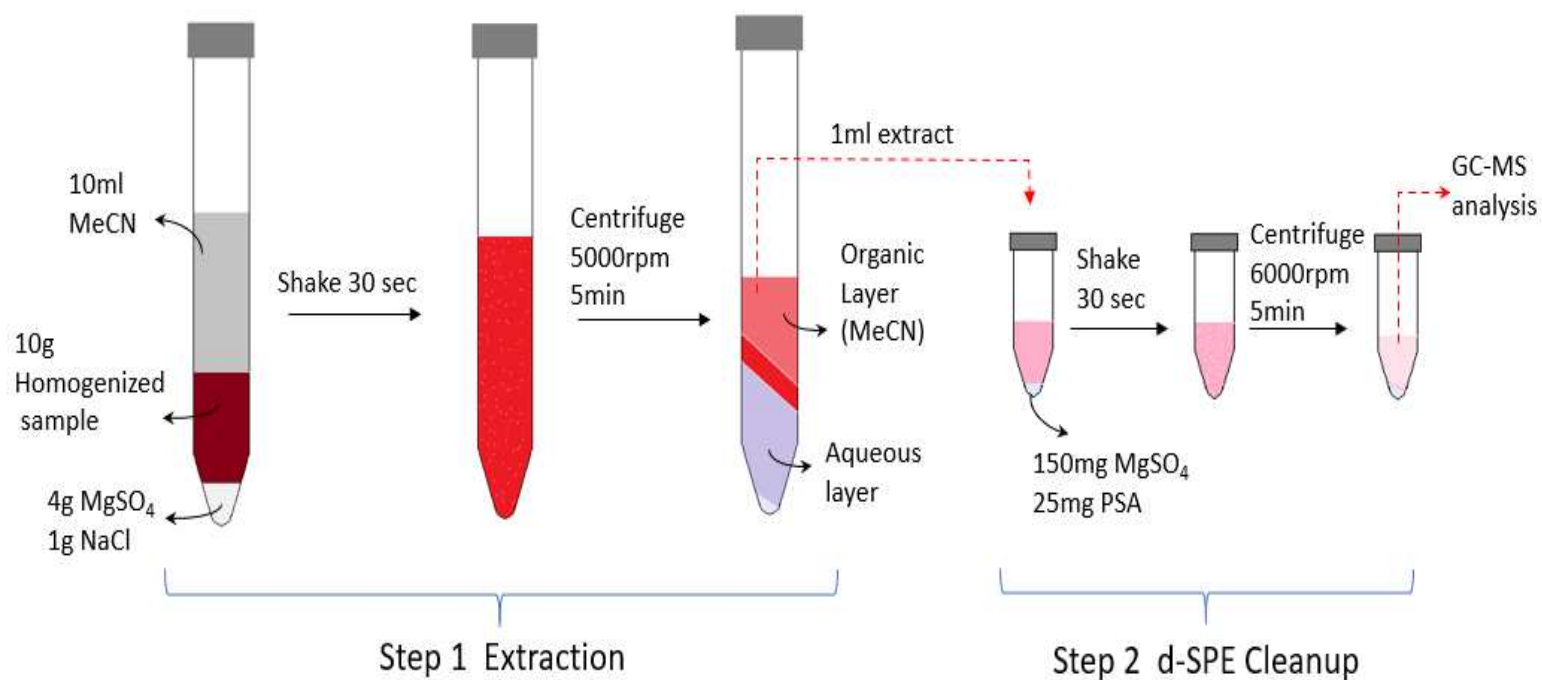
Tools

About 6,28,000 results (0.50 seconds)



QuEChERS is a solid phase extraction method for detection of pesticide residues in food. The name is a portmanteau word formed from "quick, easy, cheap, effective, rugged, and safe".

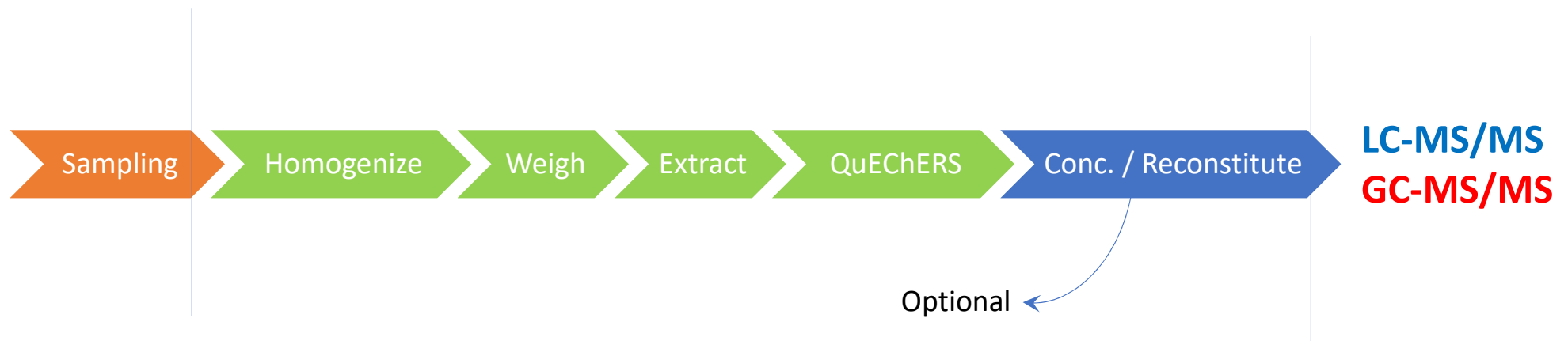
QuEChERS steps



QuEChERS - Chemicals

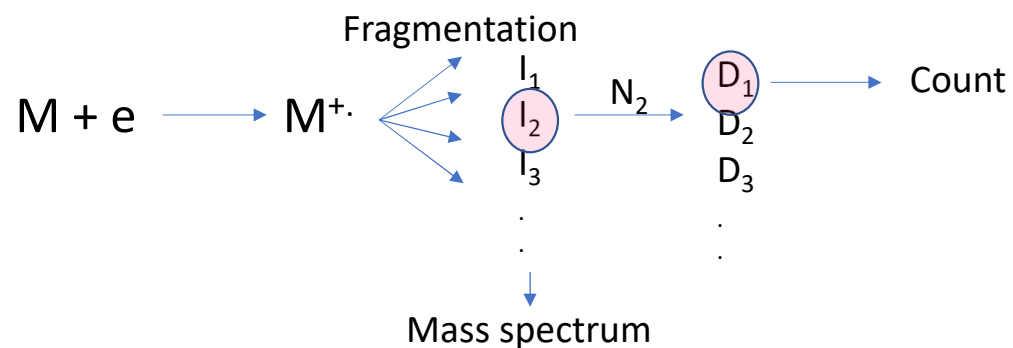
- Water is added to wet the sample, for better penetration of the extraction solvent (ACN) into the matrix. Water and ACN are miscible.
- MgSO_4 – Dissolves in water and brings about phase separation between water and ACN. Residues shift to ACN.
- Cleanup chemicals:
 - Primary Secondary Amine (PSA) – removes acidic interferences
 - C-18 sorbent – removes fatty acid interferences
 - Graphitized carbon black (GCB) – removes pigments (might affect analysis of planar pesticides)
- Careful optimization of the amounts of the cleanup chemicals are required for each matrix
- Buffering can be introduced to improve stability of pH sensitive residues

Pesticide analysis workflow

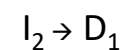


MS/MS – Multiple Reaction Monitoring

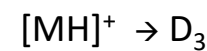
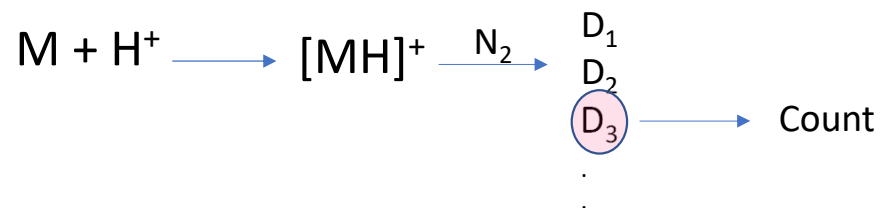
GC MS/MS



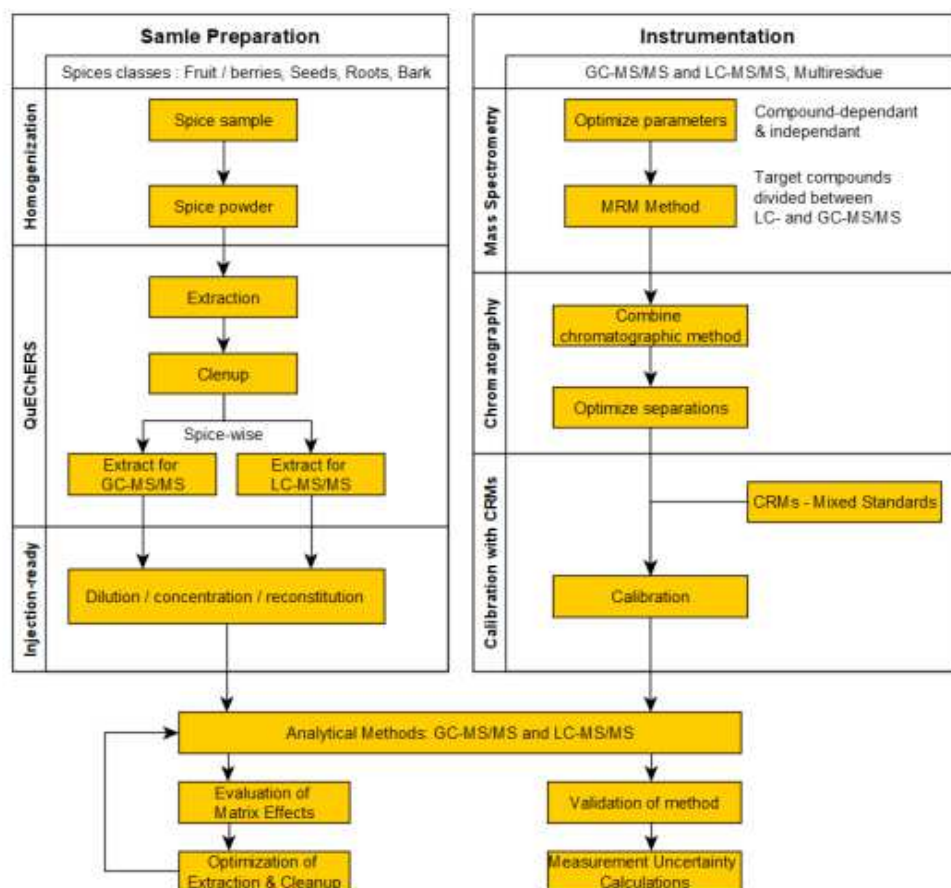
“Transitions”



LC MS/MS



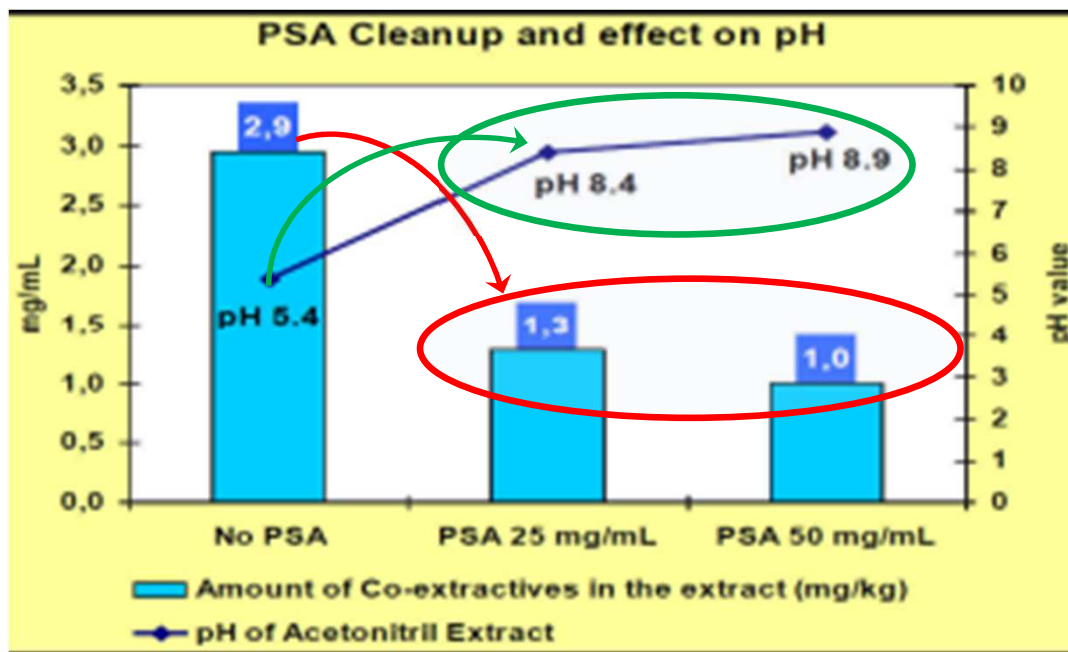
General scheme for method development



PRACTICAL QuEChERS

CHEMICAL ASPECTS

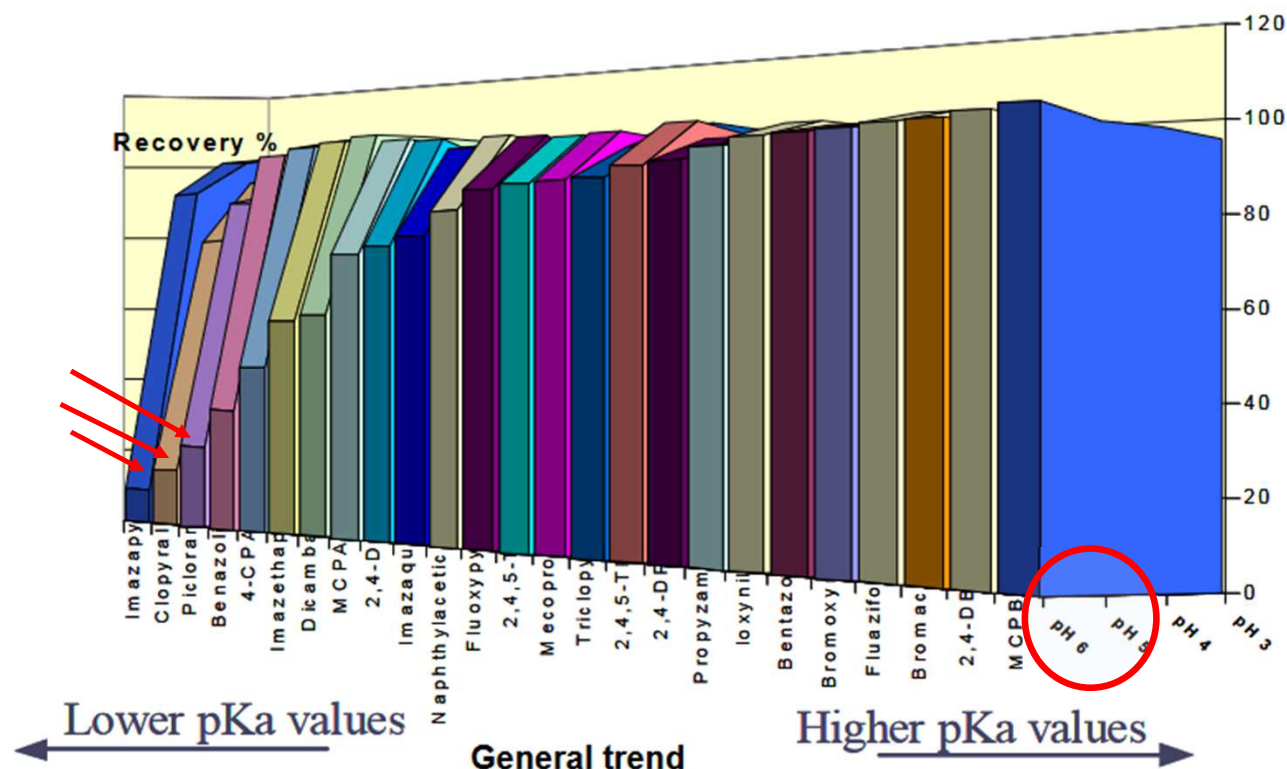
Effect of QuEChERS on extract pH



Validation of a Simple and Rapid Multiresidue Method (QuEChERS) and its Implementation in Routine Pesticide Analysis, Anastassiades, Scherbaum and Bertsch, Poster presented at the MGPR Symposium (May 2003)

Extract pH and pesticide stability

Acidic Pesticides: Matrix Apple, LC-MS/MS, n=2 Level 0.1 mg/kg



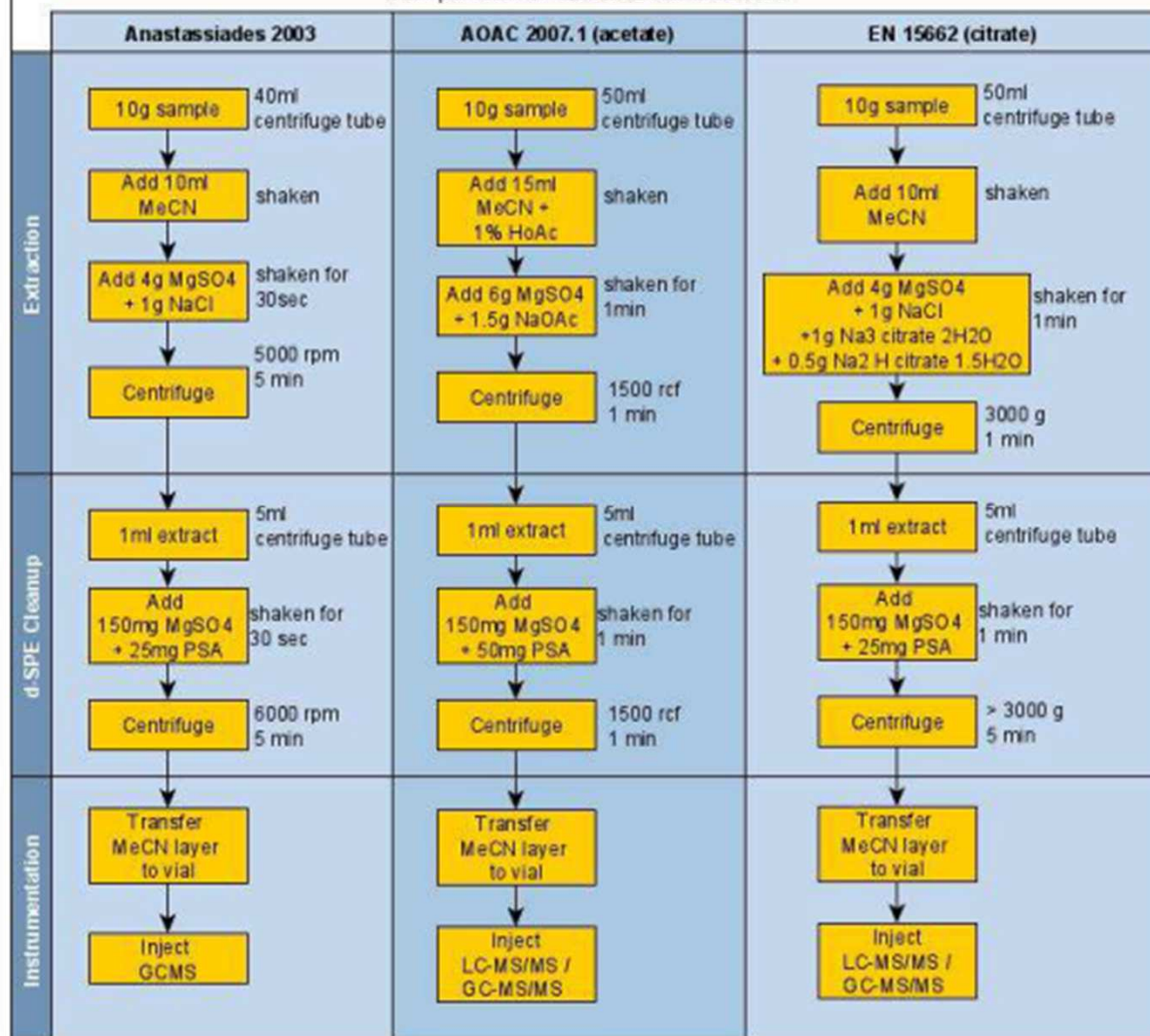
pKa is the pH above which pesticide will exist in ionized form

At high PSA loading, pesticides with low pKa values become unstable:

Use of Buffers required

Validation of a Simple and Rapid Multiresidue Method (QuEChERS) and its Implementation in Routine Pesticide Analysis, Anastassiades, Scherbaum and Bertsch, Poster presented at the MGPR Symposium (May 2003)

Comparison of QueChERS Methods



Buffers helped stabilize the pH of extract, protecting residues with less pH stability:

Original QuEChERS 2003: **No buffers**

AOAC 2007: **Acetate** buffers

EN 15662 (EU): **Citrate** buffers

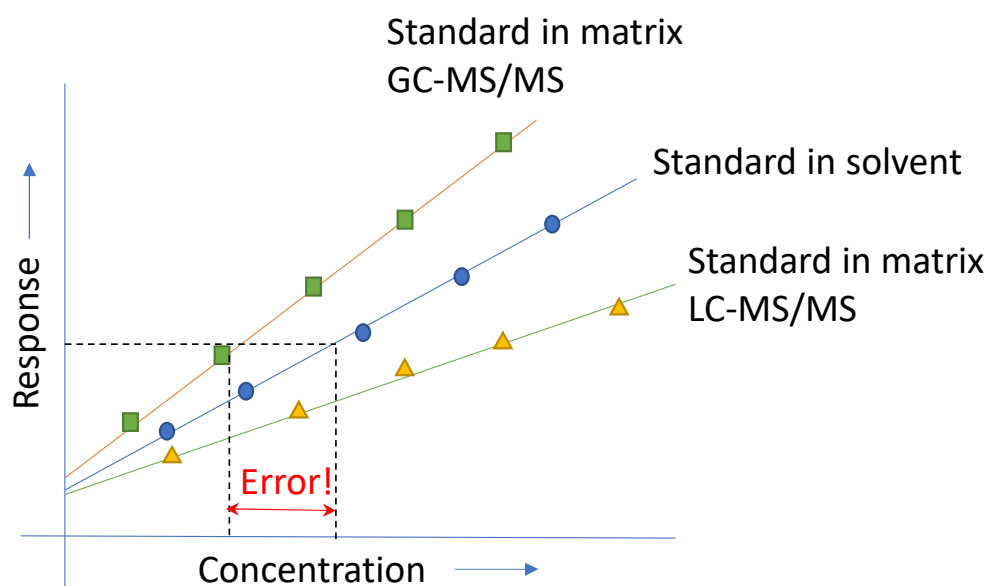
RESIDUE ANALYSIS

MATRIX EFFECTS

Matrix Effects

- Arise from the difference in behavior of the target analytes in the matrix extract as compared to that in the solvent
- Spices pose significant matrix effects (MEs) in both GC-MS/MS and LC-MS/MS analysis
- ME is usually
 - Suppressive in LC-MS/MS
 - Enhancing in GC-MS/MS
- ME has to be accounted for to obtain good results

Difference in ME in LC- and GC-MS/MS



The calibration curve

Usually,

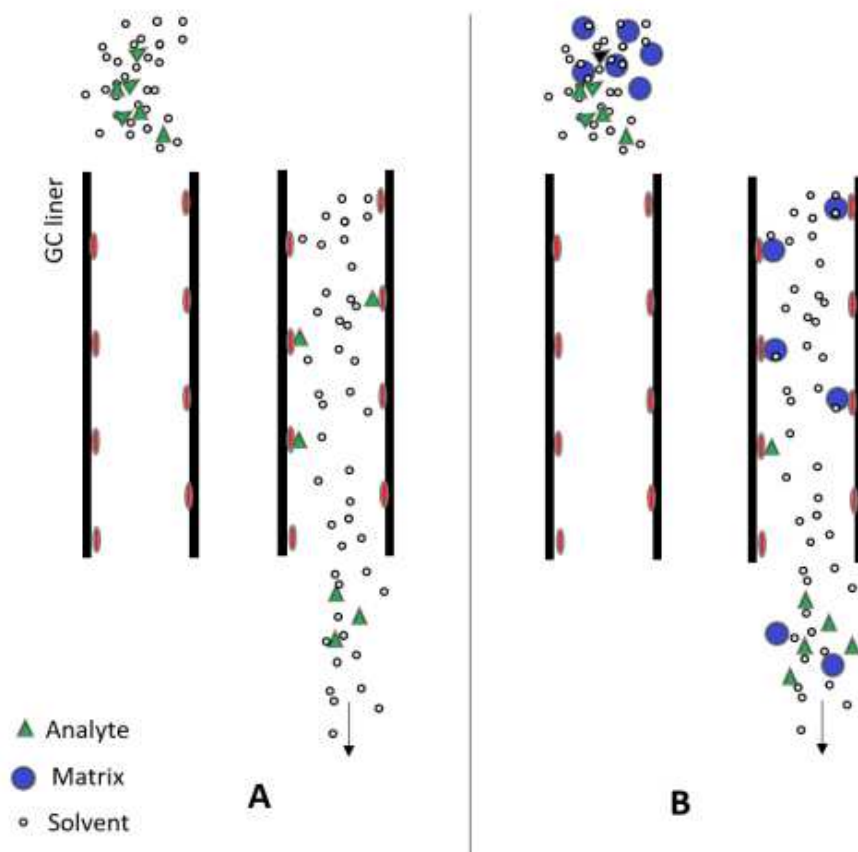
- Matrix **suppression** in **LC**-MS/MS
- Matrix **enhancement** in **GC**-MS/MS

We can't use solvent calibration curve for analytical work

Matrix-matched calibration curve required

Problem: availability of **blank matrices**

Origin of ME in GC-MS/MS

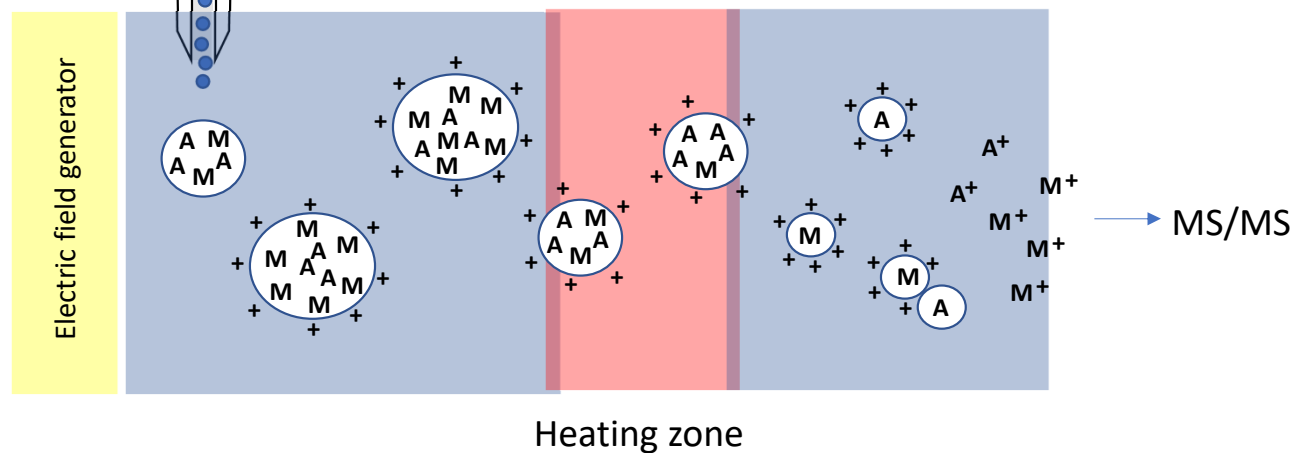


A – Injection of the analyte in solvent, **B** – injection of analyte in a matrix containing interfering coextractives

Origin of ME in LC-MS/MS

From HPLC

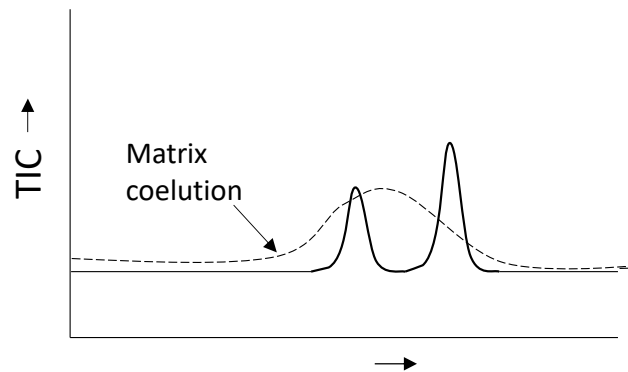
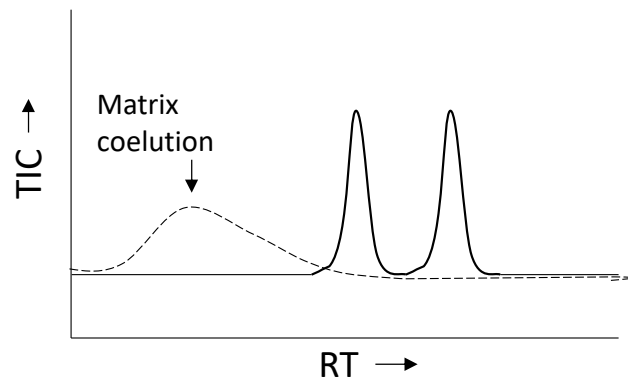
Matrix molecules inhibit
proton pickup by analyte by
competition



Matrix effect in LC-MS/MS arises in the **ionization probe**:

- When the analyte is present in solvent, it has ready access to protons in solvent and can get ionized easily
- When present in matrix extract, there is **competition between analyte and matrix compounds** for protons
- Results in matrix suppression

Coelution issues



Coelution can affect ion ratio,
qualitative errors

Adjust **chromatography** / optimize
cleanup

RESIDUE ANALYSIS

IN SPICES

Dehydration factor

Special case for spices:

- Post-harvest drying is usually involved in spices
- Residue undergoes concentration, but overall risk does not change (same amount of residue)
- A **dehydration factor** is required to convert the MRL of fresh commodity to dry form

$$DF = \frac{1}{(1 - \frac{W}{100})}$$

Where W is the % water content in spices
Assuming ~10% moisture, DF = 10

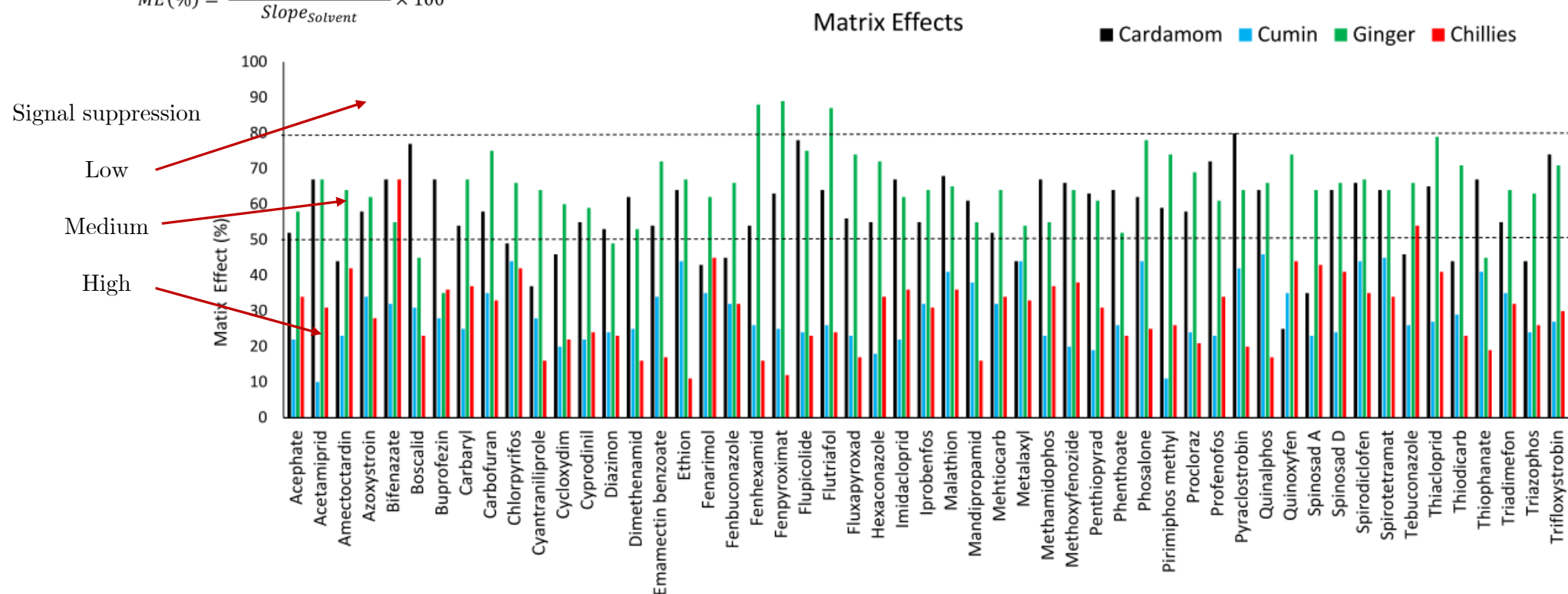
FSSAI MRLs incorporate DF in some cases.

E.g., MRL of dried chilli = $10 \times$ MRL fresh chilli for most pesticides

Matrix effects – UPLC-MS/MS

SPICES BOARD DATA

$$ME(\%) = \frac{Slope_{matrix-matched}}{Slope_{Solvent}} \times 100$$

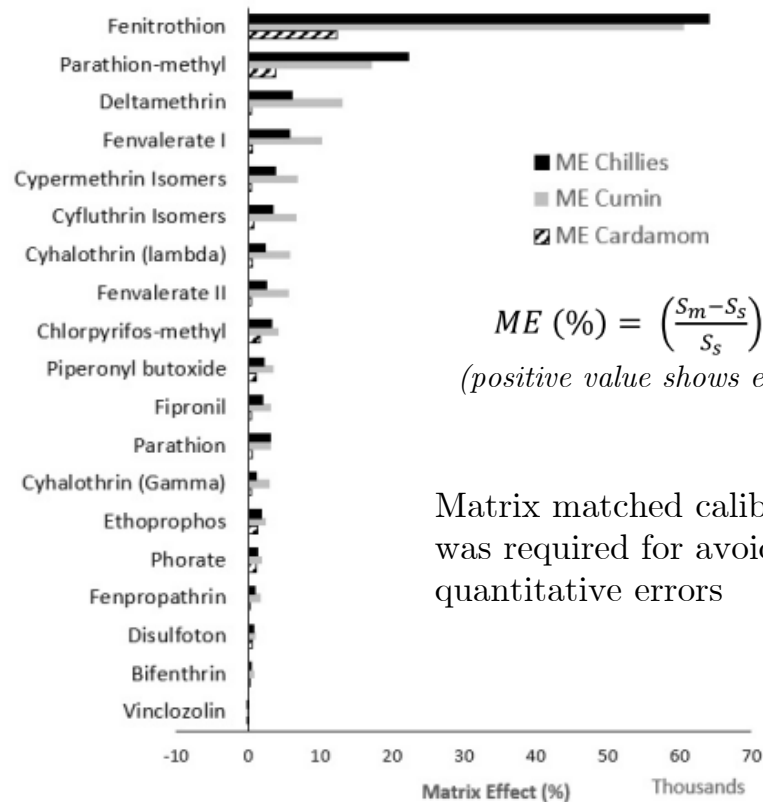
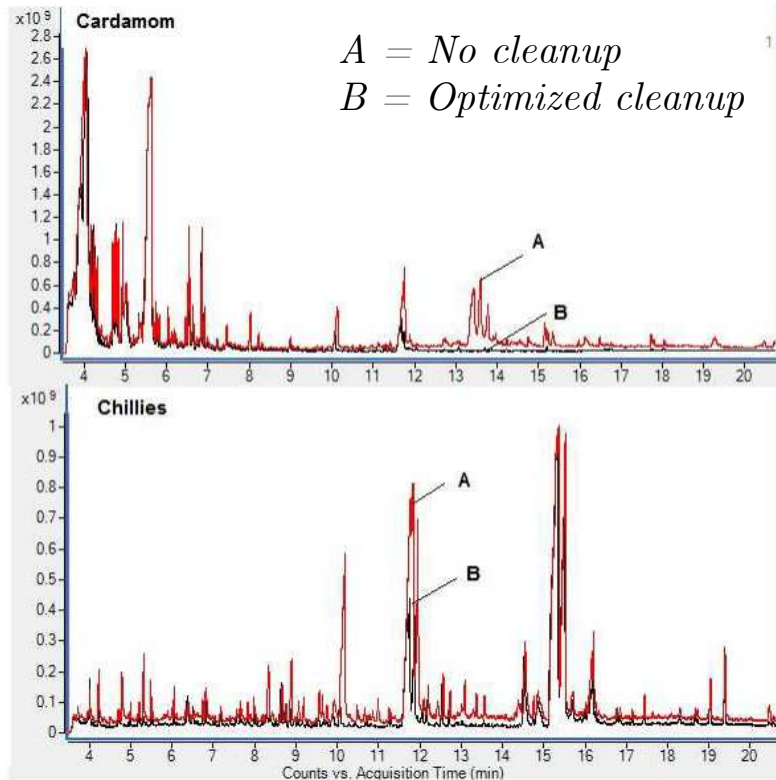


Matrix-matched calibration is required to avoid quantitative errors

Matrix effects – GC MS/MS

SPICES BOARD DATA

*Cleanup reduced
interferences at medium to
high retention times*



$$ME (\%) = \left(\frac{S_m - S_s}{S_s} \right) \times 100$$

(positive value shows enhancement)

Matrix matched calibration
was required for avoiding
quantitative errors

More to learn..

Next session 17th June 2023

- ☒ (1) Introduction to instrumentation: LC-MS/MS, 03-Jul-23
- ☒ (2) Introduction to instrumentation: GC-MS/MS, 10-Jul-23
- ☒ (3) Modern pesticide residue analysis - Introduction, 17-Jul-23
- ☐ (4) Advanced pesticide residue analysis, 24-Jul-23 ←
- ☐ (5) Method validation: requirements and practice, 31-Jul-23
- ☐ (6) Introduction to measurement uncertainty calculation, 7-Aug-23

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Thank you!
§ Questions?