

Use of chemometrics in experimental design for optimizing the conditions affecting the solid phase microextraction technique in GCMS analysis of pesticide residues in food samples

by

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Overview

- Introduction
- Solid phase microextraction
- Method development
- Univariate Design
- Multivariate Experimental design
- Method Validation
- Conclusion

INTRODUCTION

- The sample preparation step
- Need for sample preparation
- Concentration level of contaminants
- Extraction Methods
- Current trend
- Combination of multiple steps

SOLID PHASE MICROEXTRACTION

- It eliminates the need for solvents
- Efficient, effective and simple solvent-free sample preparation technique
- It offers the benefit of short sample preparation step and small sample volume
- Extraction from solid, liquid or gaseous samples

- Arthur, C. L., & Pawliszyn, J. (1990). *Anal. Chem.*, 62, 2145-2148

SOLID PHASE MICROEXTRACTION (SPME)

- It employs a chemically inert fused-silica optical fiber or metal alloys (Fiber SPME) coated with a thin film of polymeric materials
- It involve 2 steps: Partitioning of analytes and the desorption of the concentrated extracts into the analytical instrument.

SPME

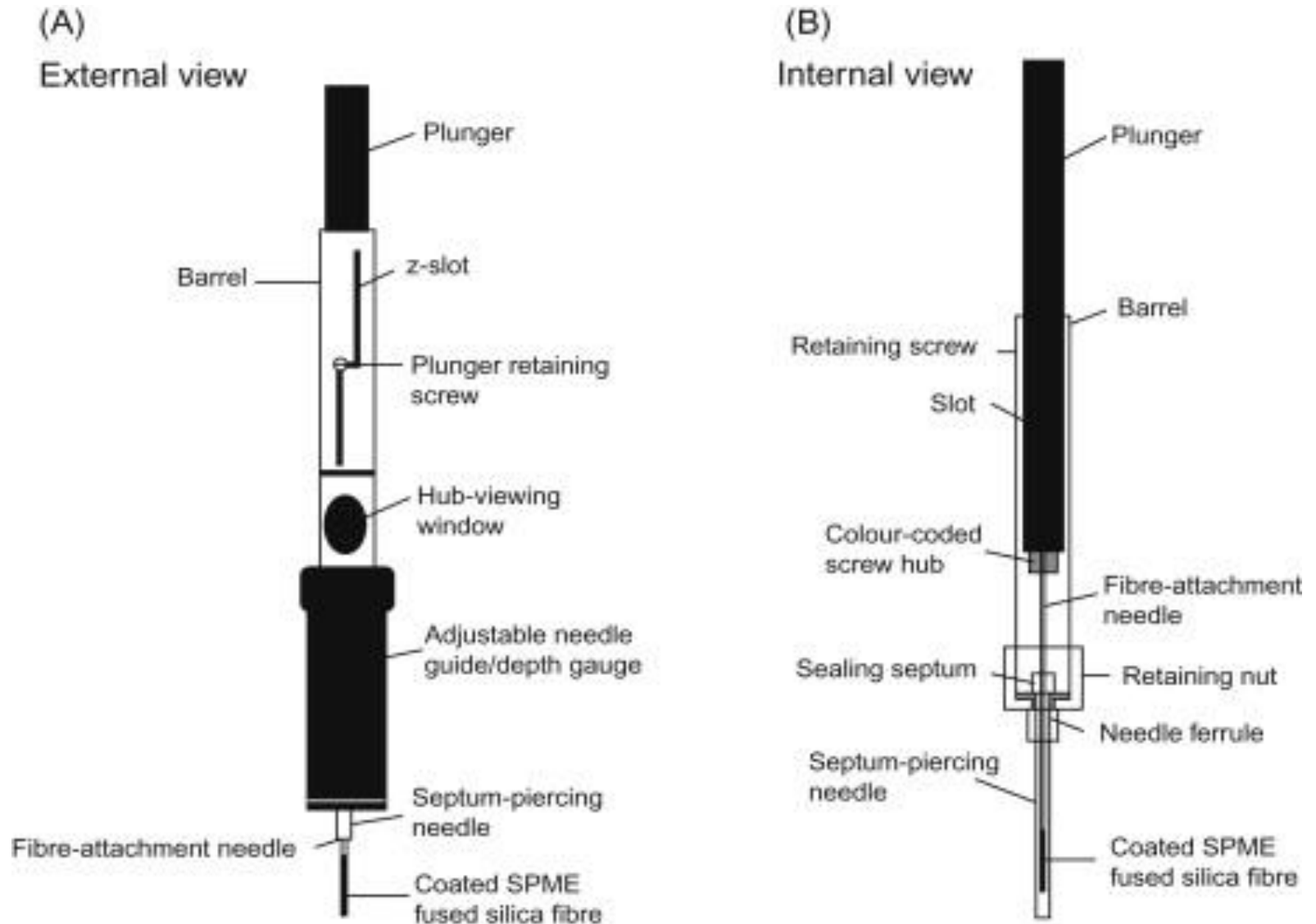


Fig 1: Manual SPME fiber holder

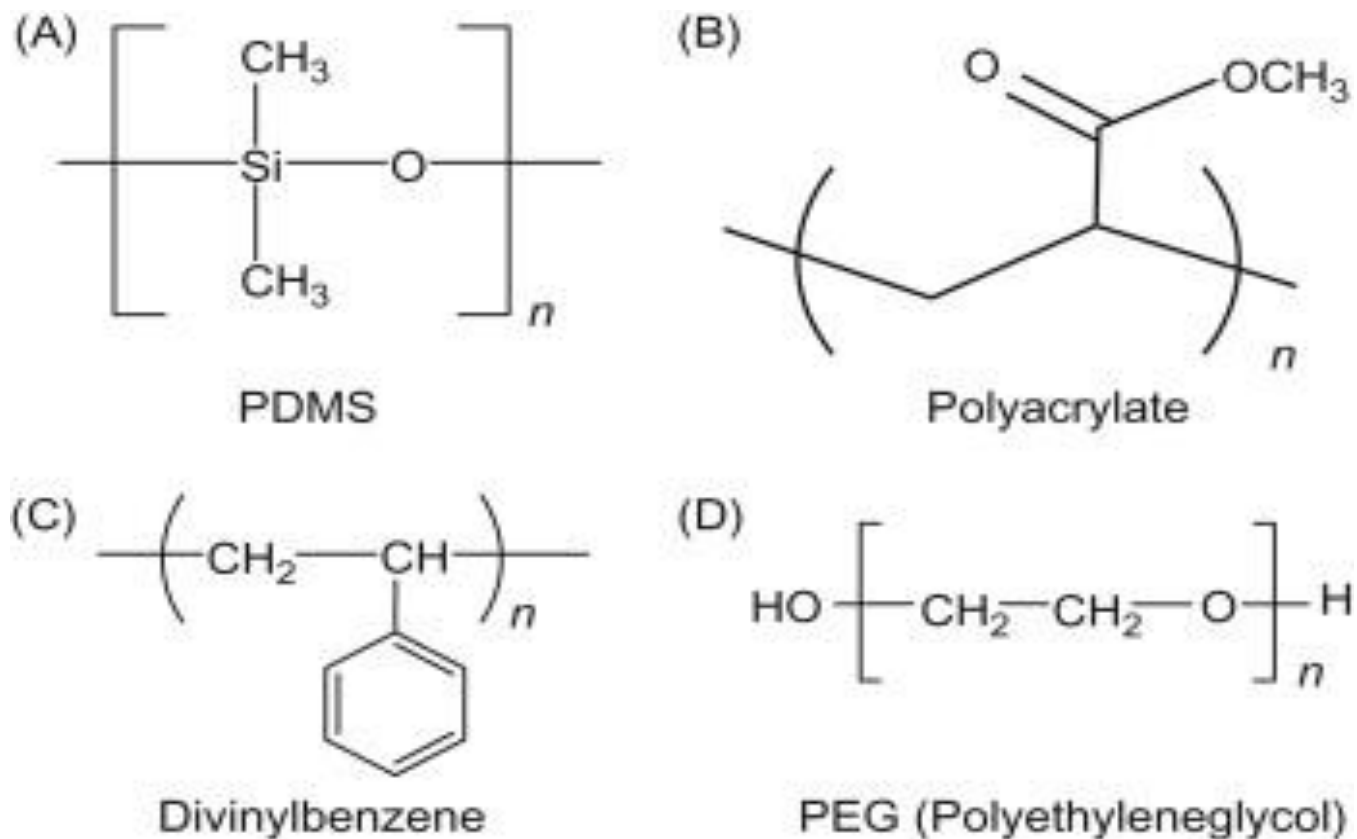


Fig 2: Chemical structure of commercial SPME coatings

- Shirey, R. E. (2012). In P. Janusz (Ed.), *Handbook of Solid Phase Microextraction* (pp. 99-133). Waltham, MA USA: Elsevier

SPME extraction modes

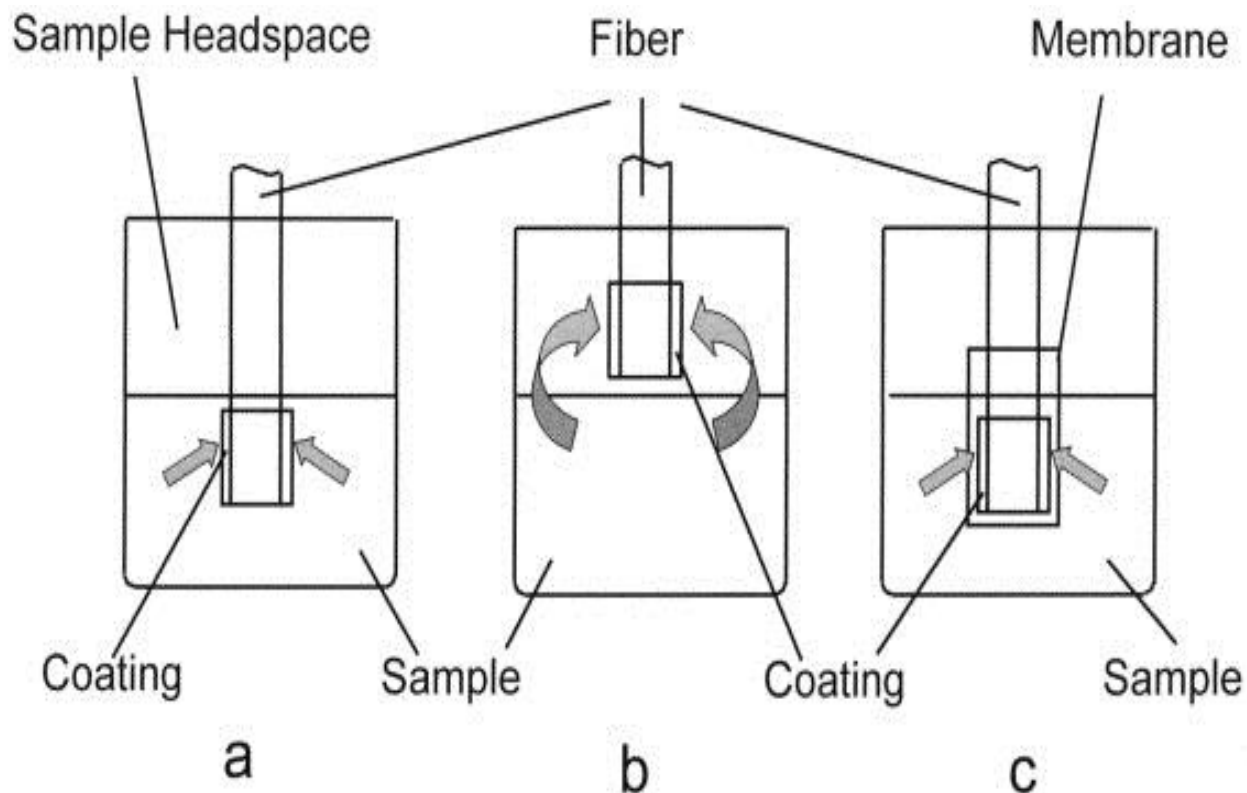


Fig 3: (a) Direct Immersion(DI), (b) Headspace (HS), (c) Membrane protected(MP)

- Lord, H., & Pawliszyn, J. (2000). *J. Chromatogr. A*, 885(1-2), 153 – 193.

Advantages of SPME

- Short sample preparation time
- Wide range of analytes and sample matrices
- Consistency and highly quantifiable results
- Small volumes of sample
- Small amounts of Organic solvents
- Environment-friendly

Factors affecting SPME

1. Fiber type
2. Extraction temperature
3. Extraction time
4. Salt effect
5. pH
7. Agitation/Stirring rate
7. Dilution factor
8. Organic solvent
9. Headspace Volume
10. Desorption temperature
11. Desorption time



- Kudlejova, L., Risticovic, S., & Vuckovic, D. (2012). In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 200-249). Waltham, USA: Elsevier.

Why Experimental Design?

- Better experiment and efficient data analysis
- Univariate approach involves many experiments.
- Screening, Optimization and Quantitative Modelling
- Allows the determination of the main and interaction effects

A well designed experiment will make analysis easy.
The proper design of an experiment is often more important than the actual analysis



Sample Preparation

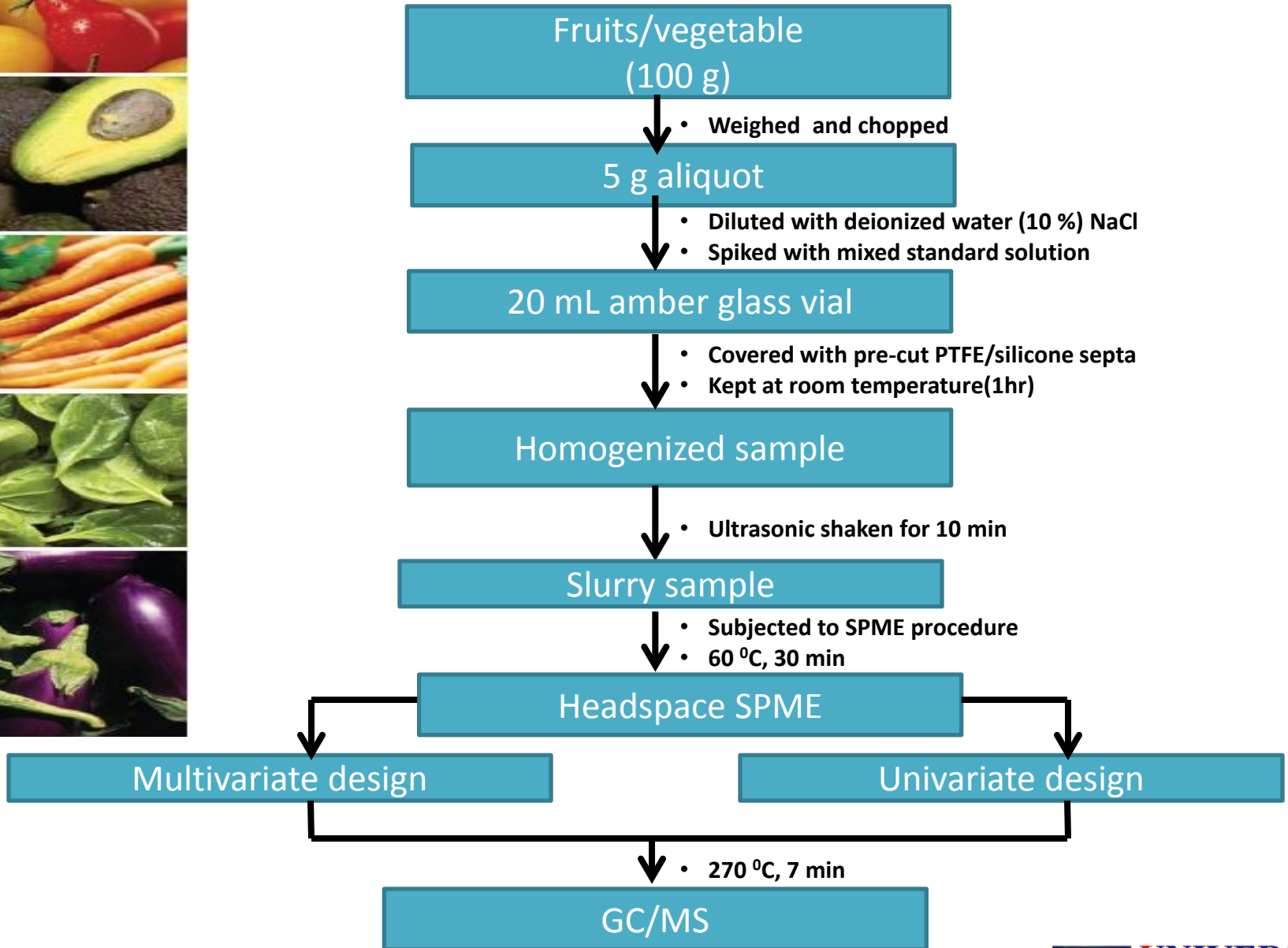


Fig 4: Sample preparation flow chart

Table 1: Univariate Optimized Parameters

| Parameters | Optimum value |
|--|------------------|
| Fiber type | 100 μ m PDMS |
| Desorption temperature ($^{\circ}$ C) | 270 |
| Desorption time (min) | 7 |
| Interface temperature ($^{\circ}$ C) | 300 |
| Column flow rate (mL/min) | 1.3 |
| Extraction time (min) | 30 |
| Extraction temperature ($^{\circ}$ C) | 60 |
| Salt addition (%) | 10 |
| pH | 8 |
| Stirring rate (rpm) | 300 |
| Solvent addition (%) | 3 |

Multivariate Design of Experiment

- Multivariate Experimental design helps to identify the significant factors that maximize the response of an experiment.
- It also helps to improve the yield of chromatographic separation by optimizing the significant factors using response surface methodology or central composite design.
- Its use is aimed to understand the effect of each factor and model the relationship between the factors and response with a minimal number of experiments carried out in an orderly and efficient manner.

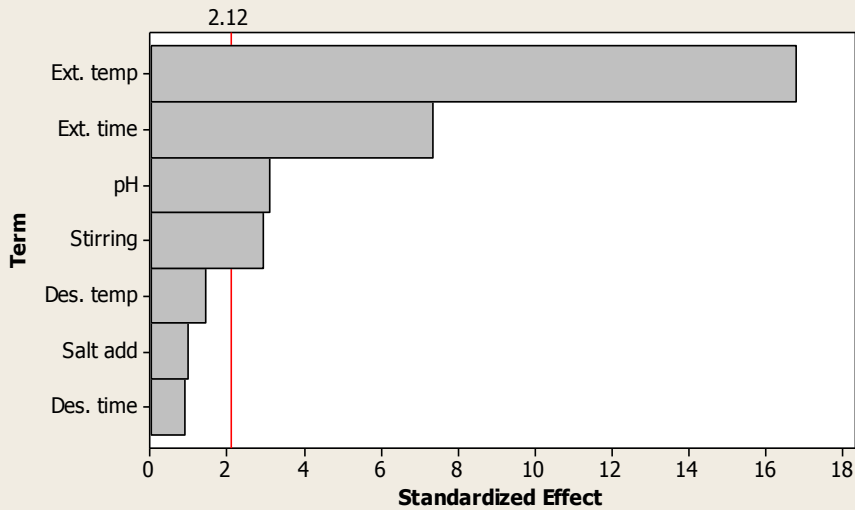
Multivariate Design of Experiment

Table 2: Factors and levels of variables

| Variables | Levels | |
|-----------------------------|---------|----------|
| | Low (-) | High (+) |
| Extraction temperature (°C) | 30 | 60 |
| Extraction time (min) | 30 | 60 |
| Salt addition (% m/v) | 5 | 10 |
| Stirring rate (rpm) | 300 | 600 |
| pH | 4 | 8 |
| Desorption time (min) | 5 | 10 |
| Desorption temperature (°C) | 250 | 270 |

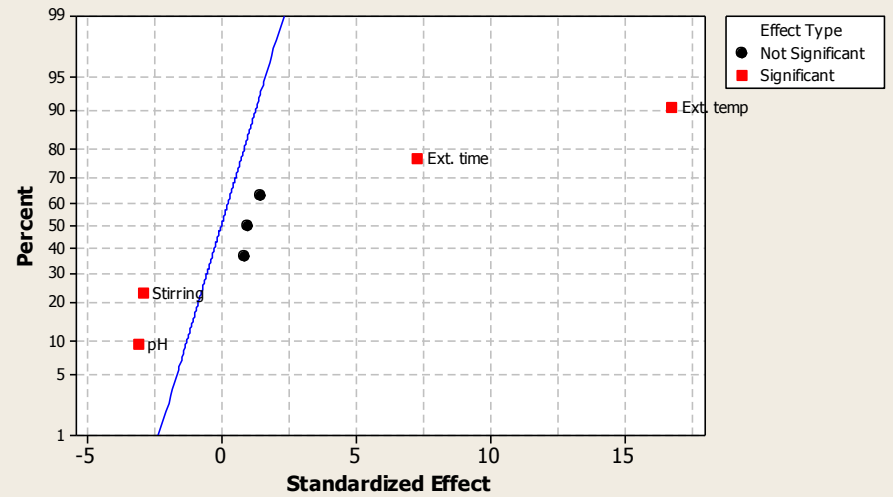
Pareto Chart of the Standardized Effects

(response is Total Peak Area, Alpha = 0.05)



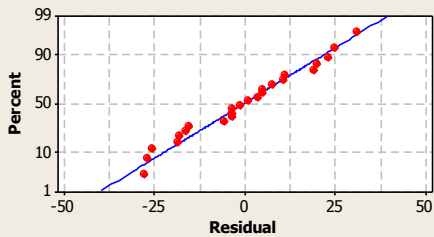
Normal Plot of the Standardized Effects

(response is Total Peak Area, Alpha = 0.05)

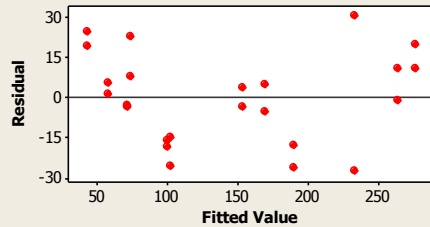


Residual Plots for Total Peak Area

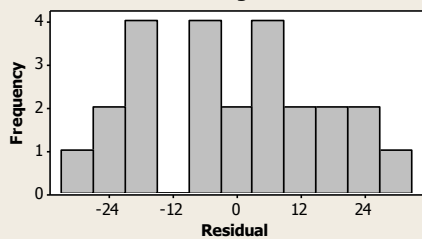
Normal Probability Plot



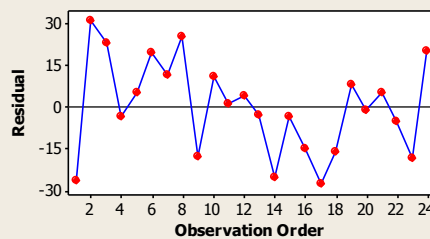
Versus Fits



Histogram



Versus Order



Main Effects Plot for Total Peak Area

Fitted Means

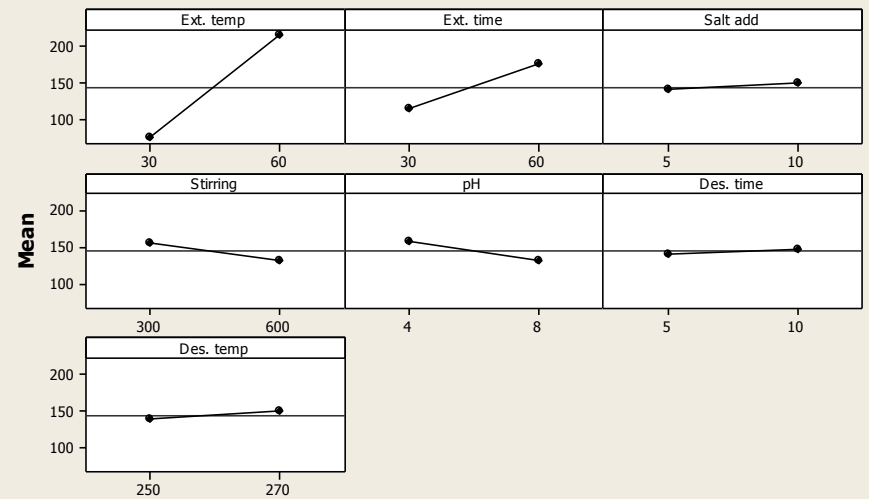


Fig 5: Multivariate experimental plots

Central Composite Design (CCD) with Full Factorial

Table 3: Factors and level used in CCD

| Variables | Level | | | Star points ($\alpha=2$) | |
|---|---------|-------------|----------|----------------------------|-----------|
| | Low (-) | Central (0) | High (+) | $-\alpha$ | $+\alpha$ |
| Extraction temp. ($^{\circ}\text{C}$) | 30 | 45 | 60 | 15 | 75 |
| Extraction time (min) | 30 | 45 | 60 | 15 | 75 |
| pH | 4 | 6 | 8 | 2 | 10 |
| Stirring rate (rpm) | 300 | 450 | 600 | 150 | 750 |

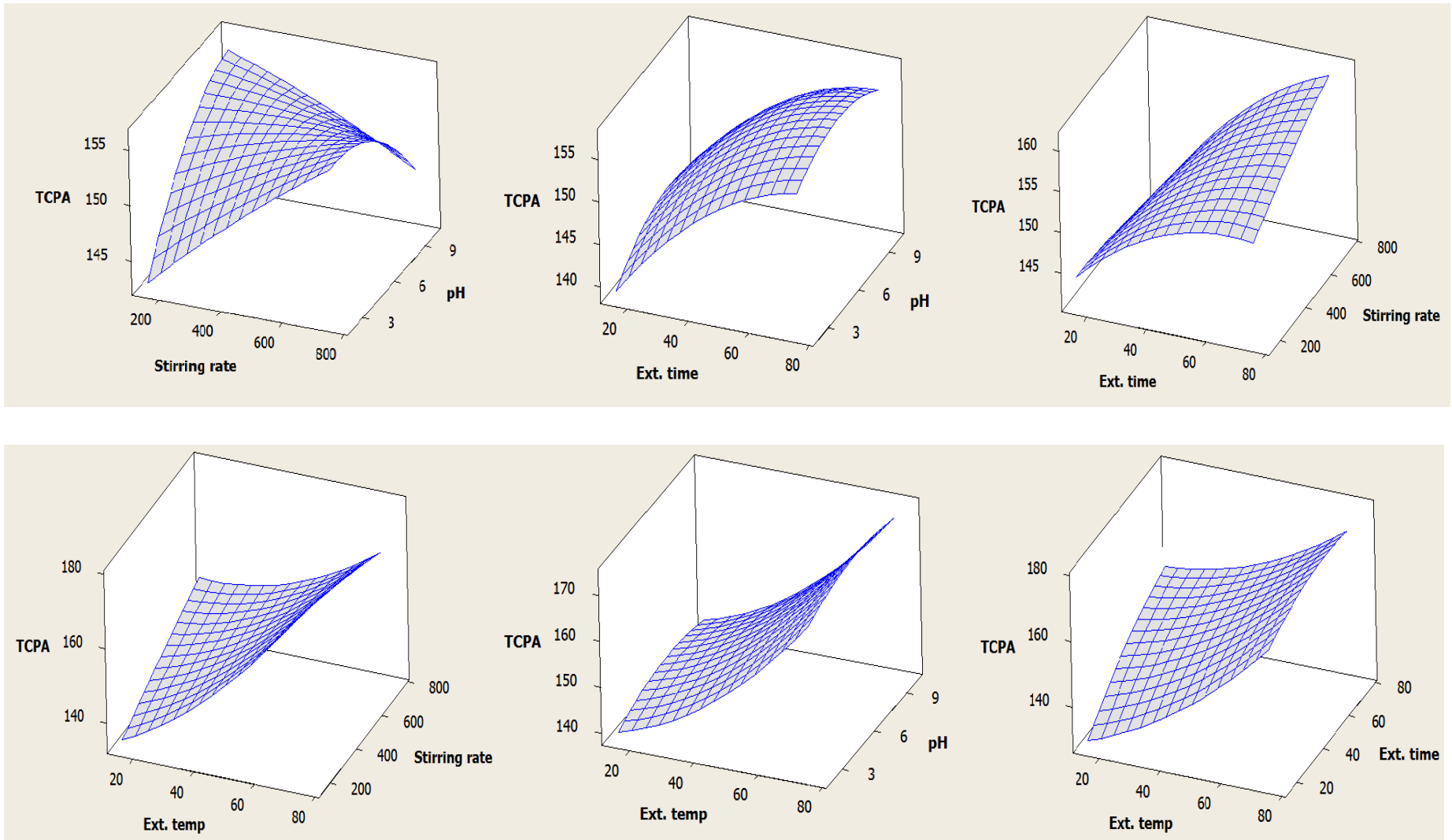


Fig 6: Desirability response surface from CCD

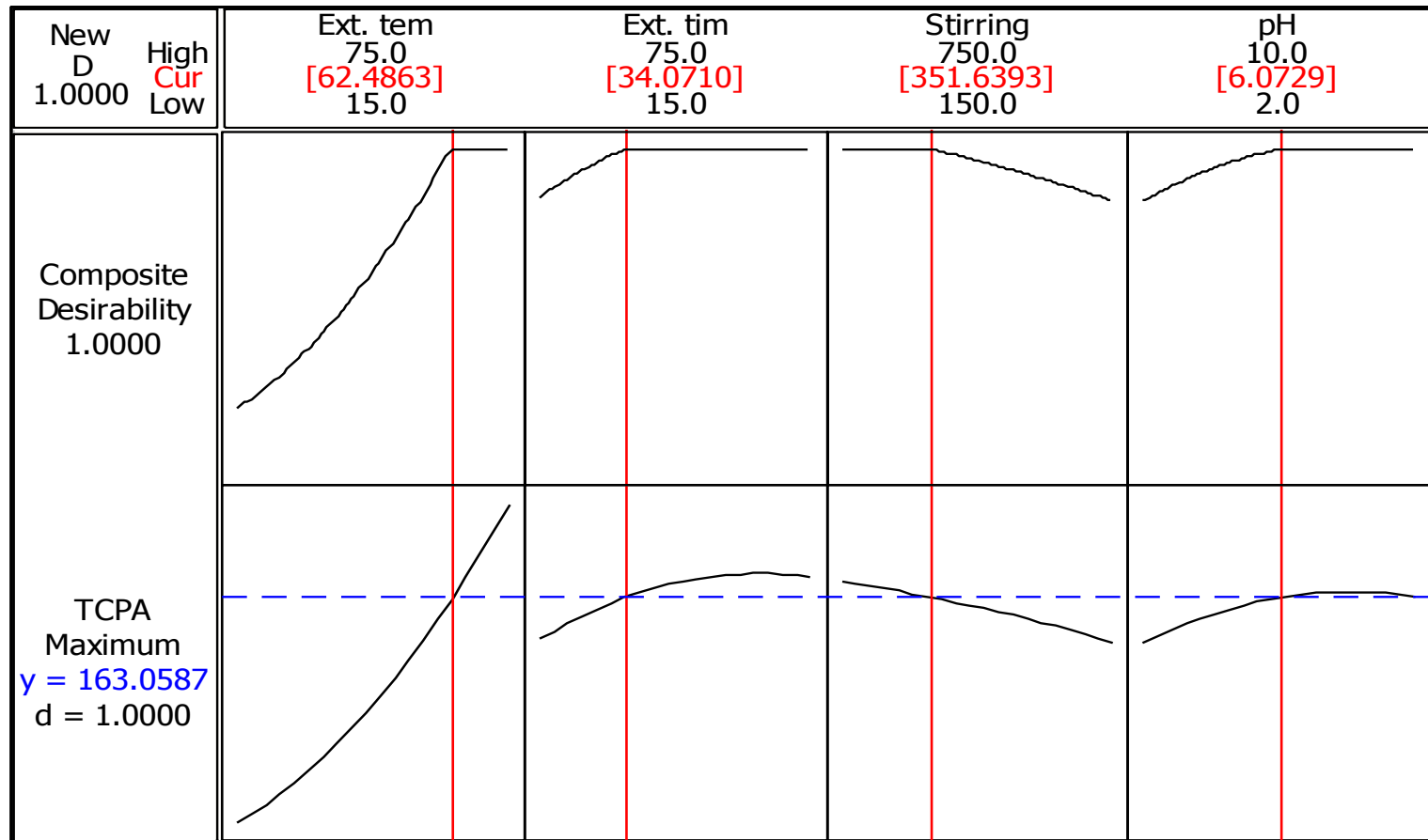


Fig 7: Response optimizer for optimized parameters

Table 4: Optimized extraction conditions

| Factors | Optimized condition |
|-----------------------------|---------------------|
| SPME fiber | PDMS |
| Extraction temperature (°C) | 65 |
| Extraction time (min) | 35 |
| Salt addition (% v/v) | 10 |
| Stirring rate (rpm) | 350 |
| pH | 6 |
| Desorption time (min) | 7 |
| Desorption temperature (°C) | 270 |

Method Validation

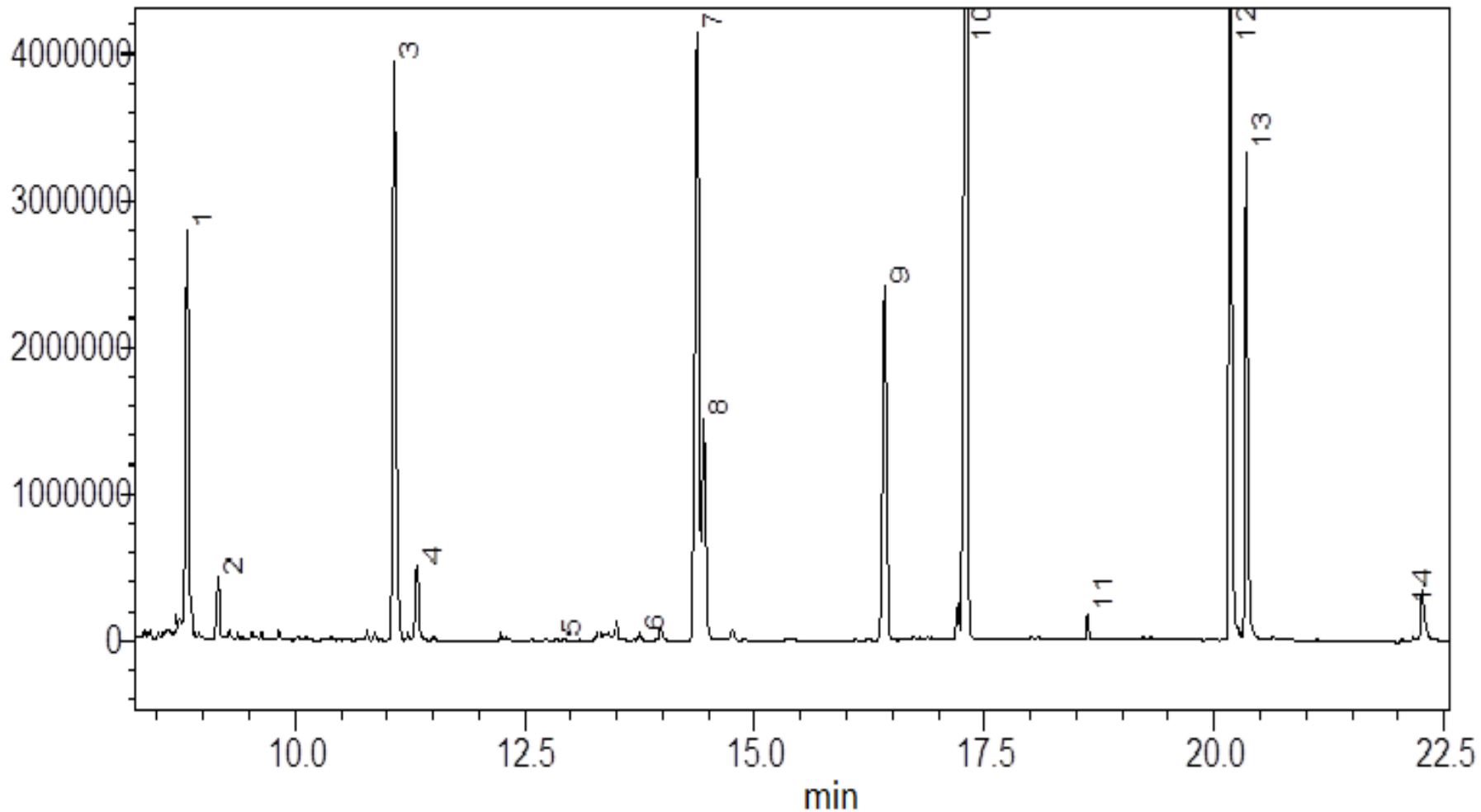


Fig 8: GC-MS Chromatogram of tomato sample spiked at 100 µg/kg

Method Validation

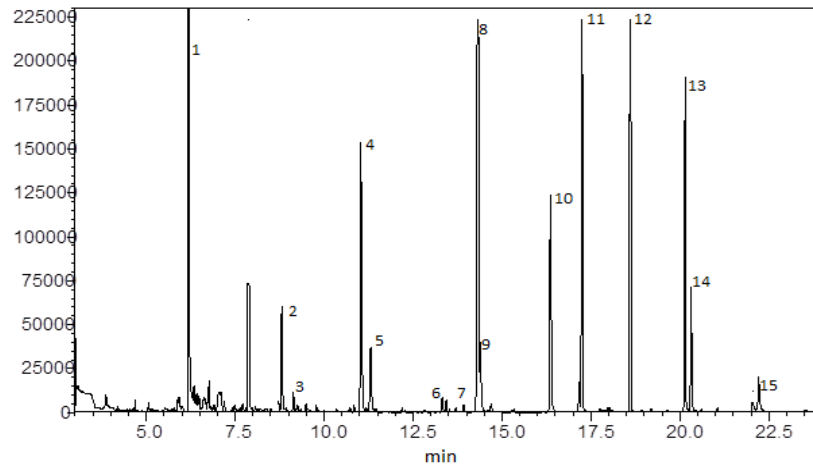
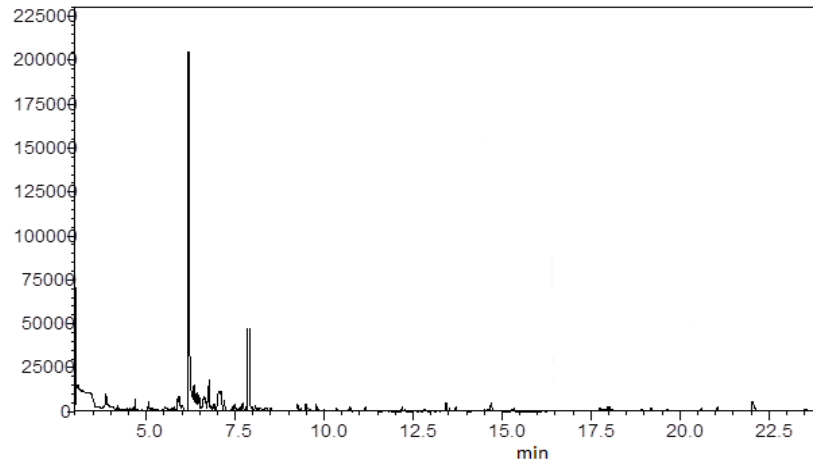


Fig 8: GC-MS Chromatogram of tomato sample spiked at 100 µg/kg

Table 5: Figures of merit of the developed method

| S/N | Analytes | LR ($\mu\text{g}/\text{kg}$) | r^2 | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | MRL (mg/kg) | Rec(RSD) (%) |
|-----|-----------------------------|-----------------------------------|--------|------------------------------------|------------------------------------|----------------------------------|-----------------|
| 1 | IS(1-chloro-3-nitrobenzene) | | | | | | |
| 2 | Fenobucarb | 2.5 – 500 | 0.9996 | 2.49 | 8.33 | 1 | 105 (2.8) |
| 3 | Ethopropop | 2.5 – 250 | 0.9986 | 0.23 | 0.77 | 0.02 | 80 (13.2) |
| 4 | Diazinone | 2.5 – 250 | 0.9948 | 0.21 | 0.68 | 0.01 | 82 (10.4) |
| 5 | Chlorothalonil | 10 – 500 | 0.9975 | 6.94 | 23.12 | 5 | 112 (8.8) |
| 6 | P-methyl | 1 – 250 | 0.9994 | 0.62 | 2.24 | 0.01 | 80 (10.5) |
| 7 | Fenitrothion | 2.5 – 200 | 0.9995 | 1.35 | 4.48 | 0.01 | 85 (12.1) |
| 8 | Chlorpyrifos | 5 – 500 | 0.9979 | 3.71 | 12.36 | 0.5 | 92 (7.4) |
| 9 | Thiobencarb | 5 – 250 | 0.9950 | 4.34 | 14.47 | 0.1 | 89 (9.5) |
| 10 | Quinalphos | 2.5 – 125 | 0.9991 | 1.94 | 6.48 | 0.05 | 86 (6.3) |
| 11 | Endosulfan I | 5 – 250 | 0.9967 | 3.91 | 13.14 | 0.05 | 87 (11.8) |
| 12 | Endosulfan II | 10 – 250 | 0.9992 | 5.19 | 17.32 | 0.05 | 87 (5.3) |
| 13 | Bifenthrin | 1 – 500 | 0.9989 | 0.99 | 3.31 | 0.3 | 91 (6.8) |
| 14 | Fenpropathrin | 1 – 50 | 0.9938 | 0.52 | 1.72 | 0.01 | 95 (7.2) |
| 15 | Permethrin | 5 – 100 | 0.9976 | 1.50 | 5.00 | 1 | 102 (11.7) |

- The developed method was used for the analysis of real tomato samples collected from 4 certified (C1–C4) and 6 uncertified (UC1–UC6) farms in Cameron highland, Malaysia.
- Note: Certified as following Good Agricultural Practice (GAP)

Table 6: Pesticide residues found in samples collected from 10 farms($\mu\text{g}/\text{kg}$)

| Analyte | C1 | C2 | C3 | C4 | UC1 | UC2 | UC3 | UC4 | UC 5 | UC6 |
|----------------|-----|-----|----------------------------------|-----|-----|-----|-----------------------------------|-----------------------------------|------|-----|
| Fenobucarb | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Ethopropop | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Diazinone | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Chlorothalonil | n.d | n.d | 15 (± 4.1) | n.d | n.d | n.d | 100 (± 9.1) | 80 (± 10.1) | n.d | n.d |
| P-methyl | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Fenitrothion | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Chlorpyrifos | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Thiobencarb | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Quinalphos | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Endosulfan I | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Endosulfan II | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Bifenthrin | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Fenpropathrin | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d | n.d |
| Permethrin | n.d | n.d | n.d | n.d | n.d | n.d | n.d | 3.5 (± 4.9) | n.d | n.d |

Maximum residue level(MRL) in Tomatoes

| Pesticide | MRL (mg/kg) |
|----------------|-------------|
| Chlorothalonil | 5 |
| Permethrin | 1 |

Linearity range ($\mu\text{g}/\text{kg}$) of the developed HS-SPME method

| Pesticides | Ret. Time (min) | Ion m/z | Range ($\mu\text{g}/\text{kg}$) | Apple r^2 | Tomato r^2 | Cucumber r^2 | Cabbage r^2 |
|------------------|-----------------|--------------|-----------------------------------|-------------|--------------|----------------|----------------------|
| Fenobucarb | 8.81 | 121, 91, 150 | 2.5–500 | 0.9975 | 0.9996 | 0.9985 | 0.9976 |
| Ethopropop | 9.13 | 158,97,139 | 2.5-250 | 0.9981 | 0.9986 | 0.9975 | 0.9979 |
| Diazinon | 11.04 | 179,137,152 | 2.5-250 | 0.9987 | 0.9948 | 0.9981 | 0.9980 |
| Chlorothalonil | 11.31 | 266,263,268 | 10-500 | 0.9987 | 0.9975 | 0.9978 | 0.9989 |
| Parathion-methyl | 12.81 | 109,79,125 | 1-250 | 0.9986 | 0.9994 | 0.9988 | 0.9964 |
| Fenitrothion | 13.70 | 125,79,109 | 2.5-200 | 0.9989 | 0.9995 | 0.9983 | 0.9952 |
| Chlorpyrifos | 14.34 | 97, 125,197 | 5-500 | 0.9980 | 0.9979 | 0.9981 | 0.9985 |
| Thiobencarb | 14.50 | 100,125,127 | 5-250 | 0.9982 | 0.9950 | 0.9984 | 0.9977 |
| Quinalphos | 16.37 | 146,118,156 | 2.5-125 | 0.9985 | 0.9991 | 0.9981 | 0.9968 |
| Endosulfan I | 17.26 | 195,207,241 | 5-250 | 0.9980 | 0.9967 | 0.9990 | 0.9976 |
| Endosulfan II | 18.61 | 195,159,207 | 10-250 | 0.9988 | 0.9992 | 0.9978 | 0.9987 |
| Bifenthrin | 20.14 | 181,166 | 1-500 | 0.9985 | 0.9989 | 0.9983 | 0.9982 |
| Fenpropathrin | 20.31 | 97,125,181 | 1-50 | 0.9976 | 0.9938 | 0.9984 | 0.9978 |
| Permethrin | 22.21 | 183,91,163 | 5-100 | 0.9969 | 0.9976 | 0.9989 | 0.9973 ²⁷ |

Figures of Merit of the Developed Method in Fruits and Vegetable Samples($\mu\text{g}/\text{kg}$)

| Pesticide | | Apple | Tomato | Pear | Grape | Cucumber | Cabbage | Lettuce |
|------------------|-----|-------|--------|-------|-------|----------|---------|---------|
| | LOD | 2.41 | 2.49 | 2.19 | 2.17 | 1.74 | 2.49 | 2.47 |
| Fenobucarb | LOQ | 8.03 | 8.33 | 7.31 | 7.22 | 5.81 | 8.33 | 8.22 |
| | MRL | 300 | 1000 | 300 | 300 | 300 | 1500 | 300 |
| | LOD | 1.31 | 0.23 | 2.51 | 1.2 | 0.35 | 0.23 | 0.34 |
| Ethopropop | LOQ | 4.36 | 0.77 | 8.36 | 4 | 1.15 | 0.77 | 1.14 |
| | MRL | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | LOD | 0.88 | 0.21 | 0.51 | 1.05 | 0.32 | 0.21 | 0.23 |
| Diazinon | LOQ | 2.92 | 0.68 | 1.84 | 3.5 | 1.05 | 0.68 | 0.77 |
| | MRL | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | LOD | 2.16 | 6.94 | 4.76 | 0.43 | 8.33 | 6.8 | 0.51 |
| Chlorothalonil | LOQ | 7.21 | 23.12 | 15.86 | 1.44 | 27.76 | 22.67 | 1.84 |
| | MRL | 1000 | 2000 | 1000 | 10 | 1000 | 1000 | 10 |
| | LOD | 0.24 | 0.62 | 0.27 | 0.22 | 0.5 | 0.53 | 0.59 |
| Parathion-methyl | LOQ | 0.79 | 2.24 | 0.89 | 0.72 | 1.65 | 1.76 | 1.96 |
| | MRL | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Figures of Merit of the Developed Method in Fruits and Vegetable Samples($\mu\text{g}/\text{kg}$)

| Pesticide | | Apple | Tomato | Pear | Grape | Cucumber | Cabbage | Lettuce |
|---------------|-----|-------|--------|------|-------|----------|---------|---------|
| Endosulfan II | LOD | 2.17 | 3.19 | 2.71 | 3.28 | 2.08 | 2.93 | 3.06 |
| | LOQ | 7.23 | 10.63 | 9.03 | 10.95 | 6.92 | 9.77 | 10.2 |
| | MRL | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Bifenthrin | LOD | 0.11 | 0.99 | 0.17 | 0.75 | 0.89 | 0.74 | 0.64 |
| | LOQ | 0.38 | 3.31 | 0.6 | 2.5 | 2.96 | 2.47 | 2.14 |
| | MRL | 300 | 300 | 300 | 100 | 300 | 100 | 2000 |
| Fenpropathrin | LOD | 0.14 | 0.52 | 0.22 | 0.55 | 0.75 | 0.47 | 0.34 |
| | LOQ | 0.47 | 1.72 | 0.74 | 1.83 | 2.5 | 1.57 | 1.13 |
| | MRL | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Permethrin | LOD | 1.01 | 1.5 | 2.03 | 1.94 | 2.42 | 1.8 | 1.65 |
| | LOQ | 3.36 | 5 | 6.78 | 6.44 | 8.05 | 6 | 5.5 |
| | MRL | 50 | 50 | 50 | 50 | 50 | 50 | 50 |

MRL maximum residue level
from European Union Data
([EU, 2005](#))

Pesticide Residues found in some Fruits and Vegetable Samples ($\mu\text{g}/\text{kg}$), triplicate

| Pesticides | Apple | Tomato | Pear | Grape | Cucumber | Cabbage | Lettuce |
|----------------|-------|--------|------|-------|----------|---------|---------|
| Fenobucarb | nd | nd | nd | nd | nd | nd | nd |
| Ethopropop | nd | nd | nd | nd | nd | nd | nd |
| Diazinon | nd | nd | nd | nd | 2.1 | nd | nd |
| Chlorothalonil | nd | 80 | nd | nd | nd | nd | nd |
| Parathion-M | nd | nd | nd | nd | nd | nd | nd |
| Fenitrothion | nd | nd | nd | nd | nd | nd | nd |
| Chlorpyrifos | 22.4 | nd | nd | nd | nd | nd | nd |
| Thiobencarb | nd | nd | nd | nd | nd | nd | nd |
| Quinalphos | nd | nd | nd | nd | nd | nd | nd |
| Endosulfan I | nd | nd | nd | nd | nd | nd | nd |
| Endosulfan II | nd | nd | nd | nd | nd | nd | nd |
| Bifenthrin | nd | nd | nd | nd | nd | nd | nd |
| Fenpropathrin | nd | nd | nd | nd | nd | nd | nd |
| Permethrin | nd | 13.5 | nd | nd | nd | nd | nd |

Conclusion

- **Cost efficient**
- **Simplicity**
- **Sensitivity**
- **Detection limits**
- **Wide range of application**

Some Recent Publications from study

- L.B.Abdulra'uf & G.H. Tan(2014): “Chemometric Study and Optimization of Headspace Solid-Phase Microextraction Parameters for the Determination of Multiclass Pesticide Residues in Processed Cocoa from Nigeria Using Gas Chromatography/Mass Spectrometry” Journal of AOAC International Vol. 97, No. 4, 2014, **1**
- L.B.Abdulra'uf & G.H. Tan(2013): “Multivariate study of parameters in the determination of pesticide residues in Apple by headspace solid phase microextraction coupled to gas chromatography mass spectrometry using experimental factorial design”, Food Chem, 141, 4344-4348

- Lukman Bola Abdulra'uf , Guan Huat Tan(2015), “Chemometric approach to the optimization of HS-SPME/GC–MS for the determination of multiclass pesticide residues in fruits and vegetables”, Food Chem, 177, 267-273



For your attention