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

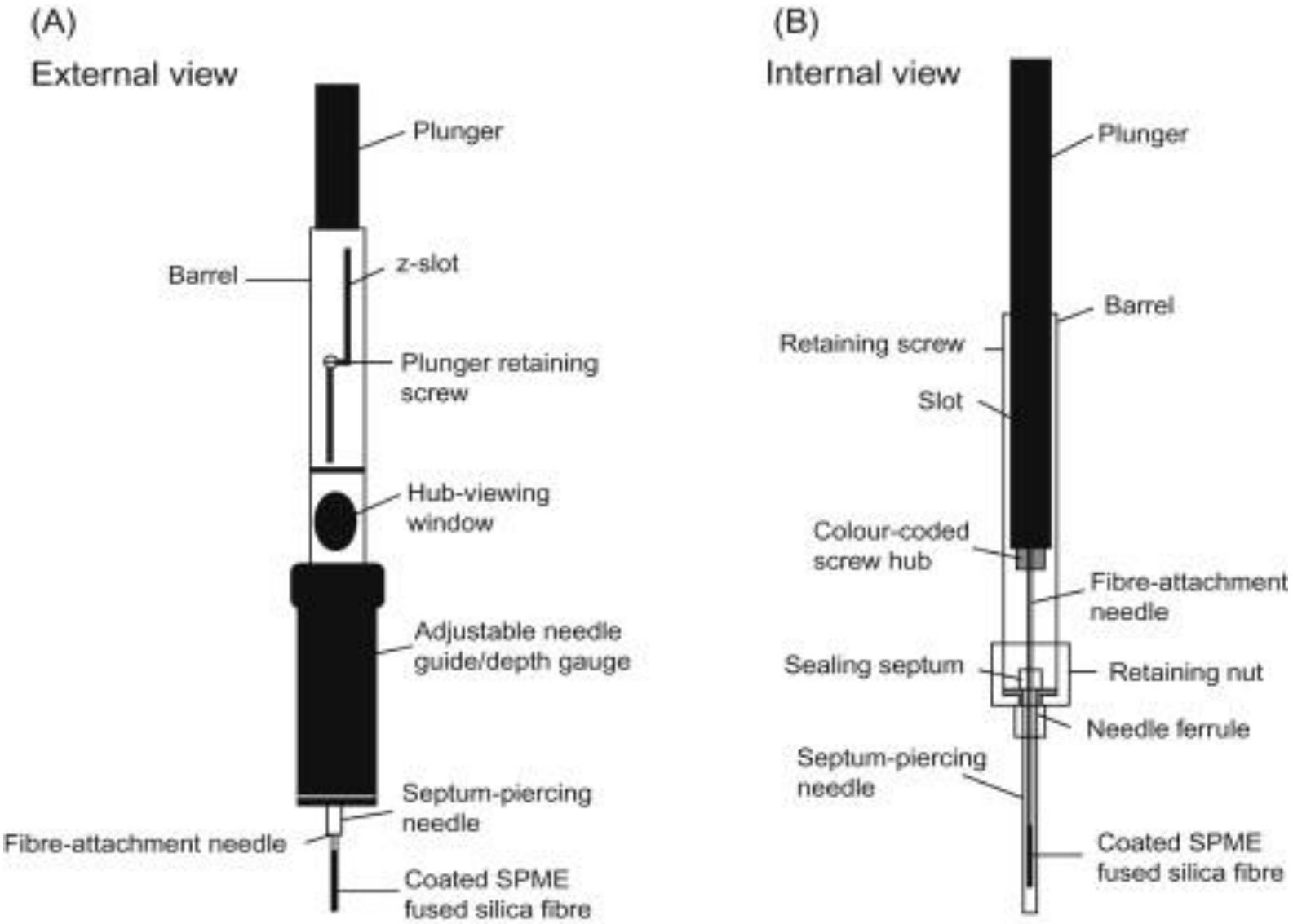

Fig 1: Manual SPME fiber holder

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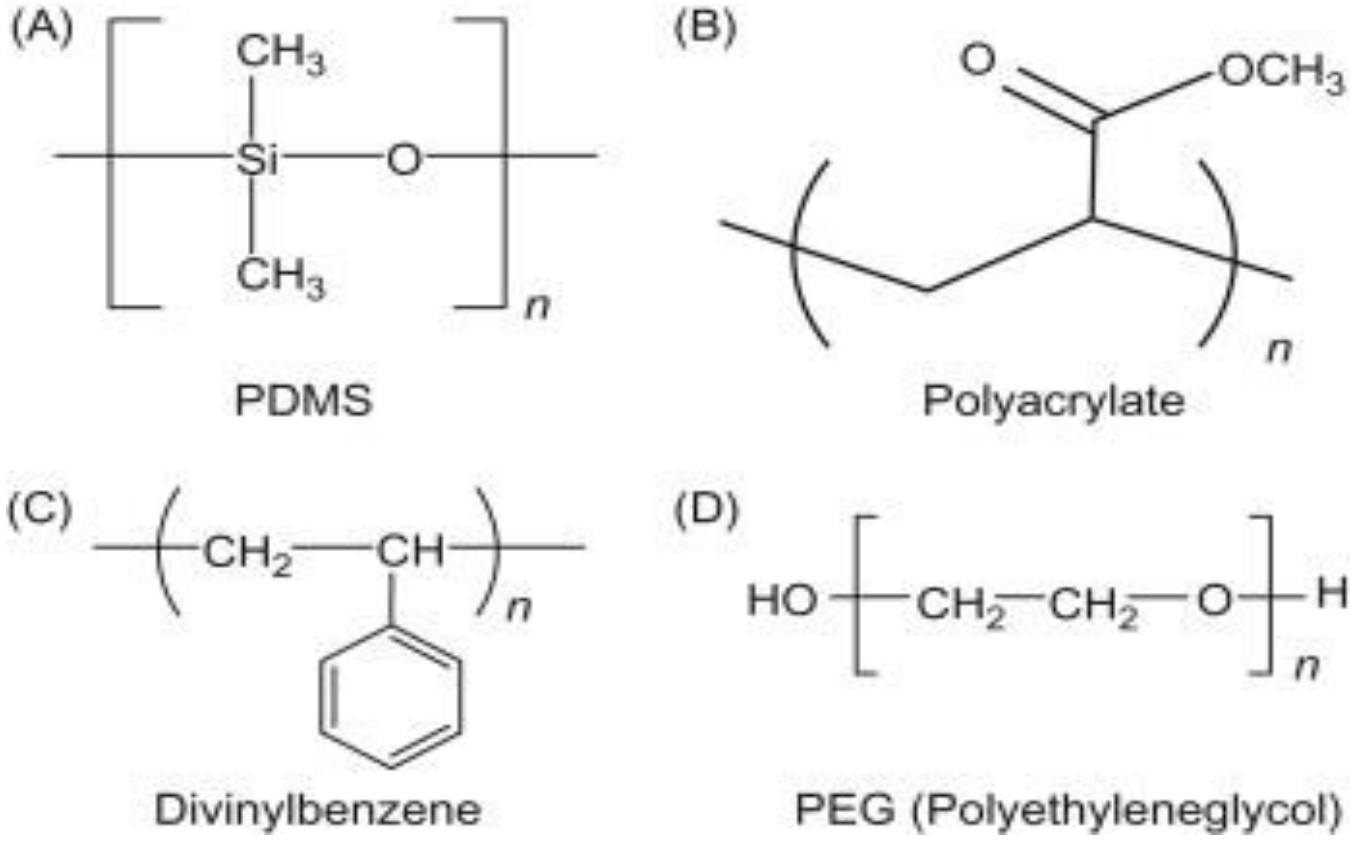


Fig 2: Chemical structure of commercial SPME coatings

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

SPME extraction modes

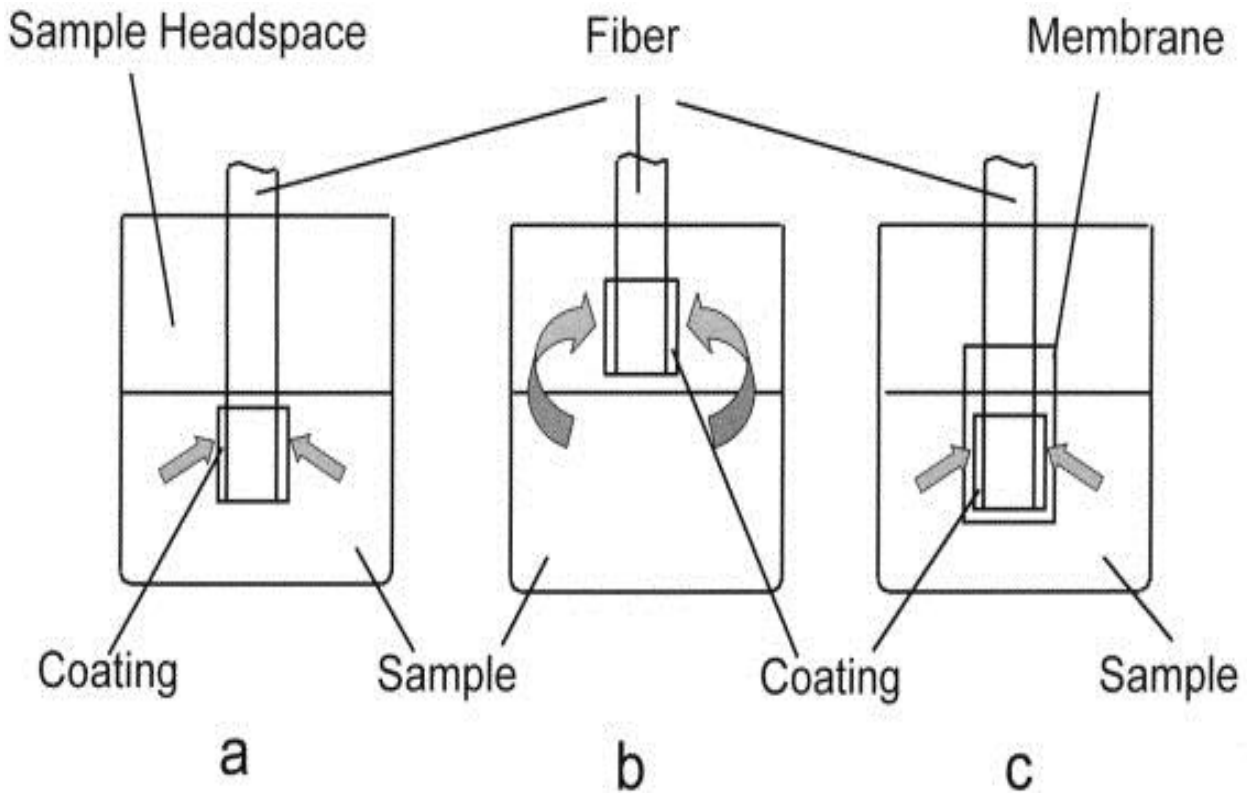


Fig 3: (a) Direct Immersion(DI), (b) Headspace (HS), (c) Membrane protected(MP)

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

Why Experimental Design?

- Better experiment and efficient data analysis
- Univariate approach involves many experiments.
- Screening, Optimization and Quantitaive 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

Fruits/vegetable (100 g)

- Weighed and chopped

5 g aliquot

- Diluted with deionized water (10 %) NaCl
- Spiked with mixed standard solution

20 mL amber glass vial

- Covered with pre-cut PTFE/silicone septa
- Kept at room temperature(1hr)

Homogenized sample

- Ultrasonic shaken for 10 min

Slurry sample

- Subjected to SPME procedure
- 60 ^{0}C, 30 min

Headspace SPME

Multivariate design

Univariate design

- 270 ^{0}C, 7 min

GC/MS

Fig 4: Sample preparation flow chart

Table 1: Univariate Optimized Parameters

Parameters	Optimum value
Fiber type	100 µm PDMS
Desorption temperature (^{0}C)	270
Desorption time (min)	7
Interface temperature (^{0}C)	300
Column flow rate (mL/min)	1.3
Extraction time (min)	30
Extraction temperature (^{0}C)	60
Salt addition (%)	10
pH	8
Stirring rate (rpm)	300
Solvent addition (%)	3

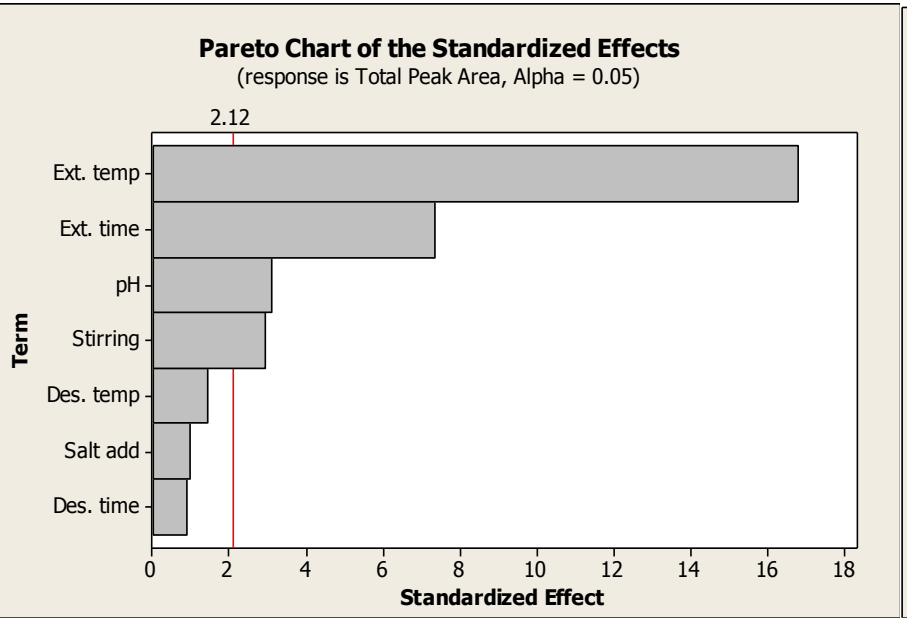
Multivariate Design of Experiment

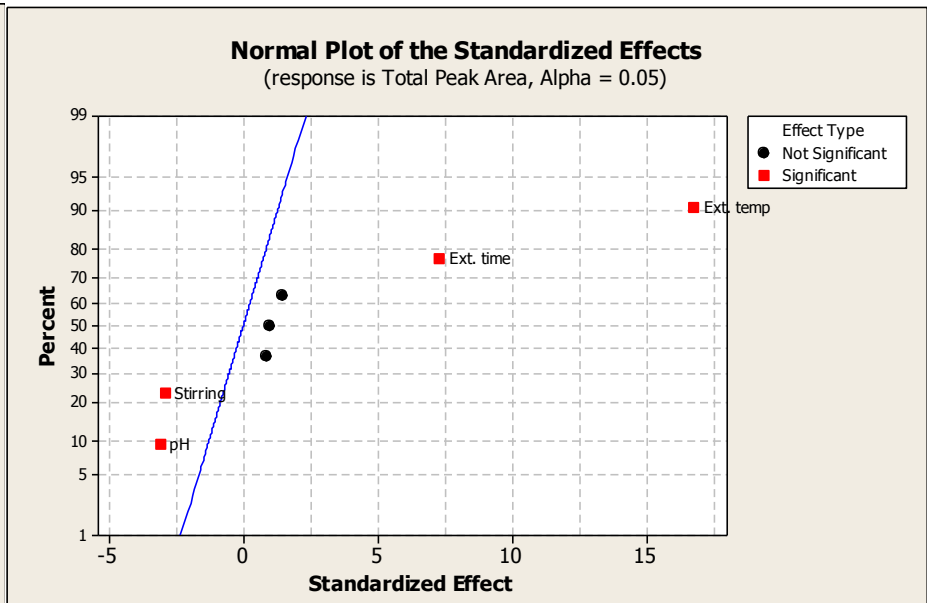
- Multivariate Experimental design helps to identify the significant factors that maximize the response of an experiment.
- It also helps to improve the yield of chromatographic separation by optimizing the significant factors using response surface methodology or central composite design.
- Its use 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.

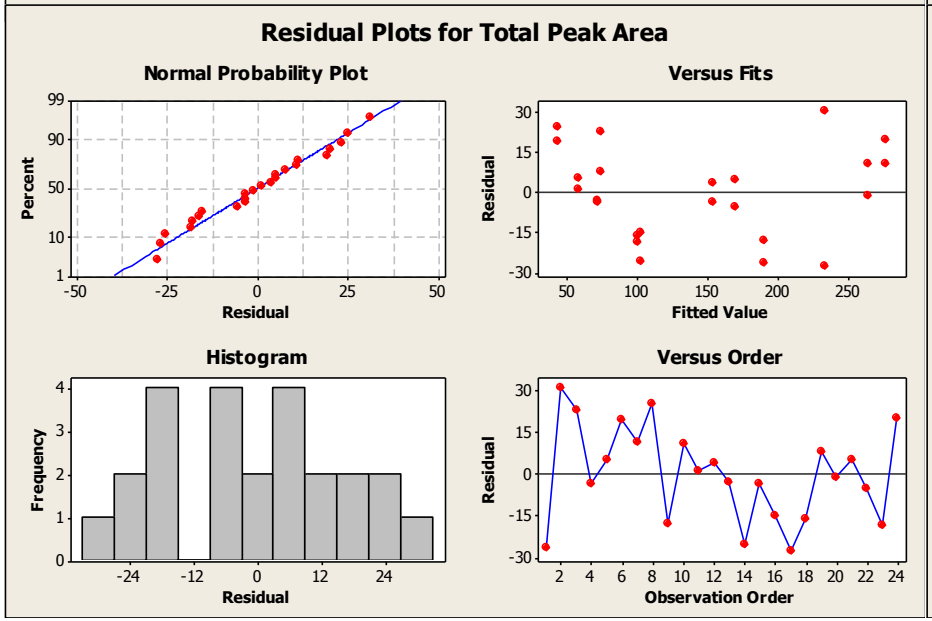
Multivariate Design of Experiment

Table 2: Factors and levels of variables

Variables	Levels	
	Low (-)	High (+)
Extraction temperature (^{0}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 (^{0}C)	250	270







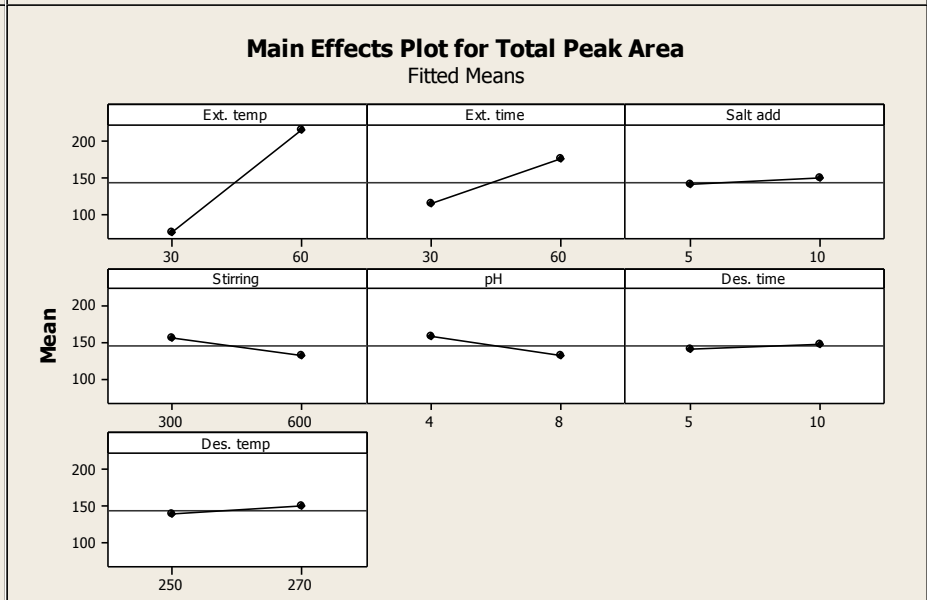


Fig 5: Multivariate experimental plots

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Central Composite Design (CCD) with Full Factorial

Table 3: Factors and level used in CCD

Variables	Level			Star points (α=2)	
	Low (–)	Central (0)	High (+)	–α	+α
Extraction temp. (^{0}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

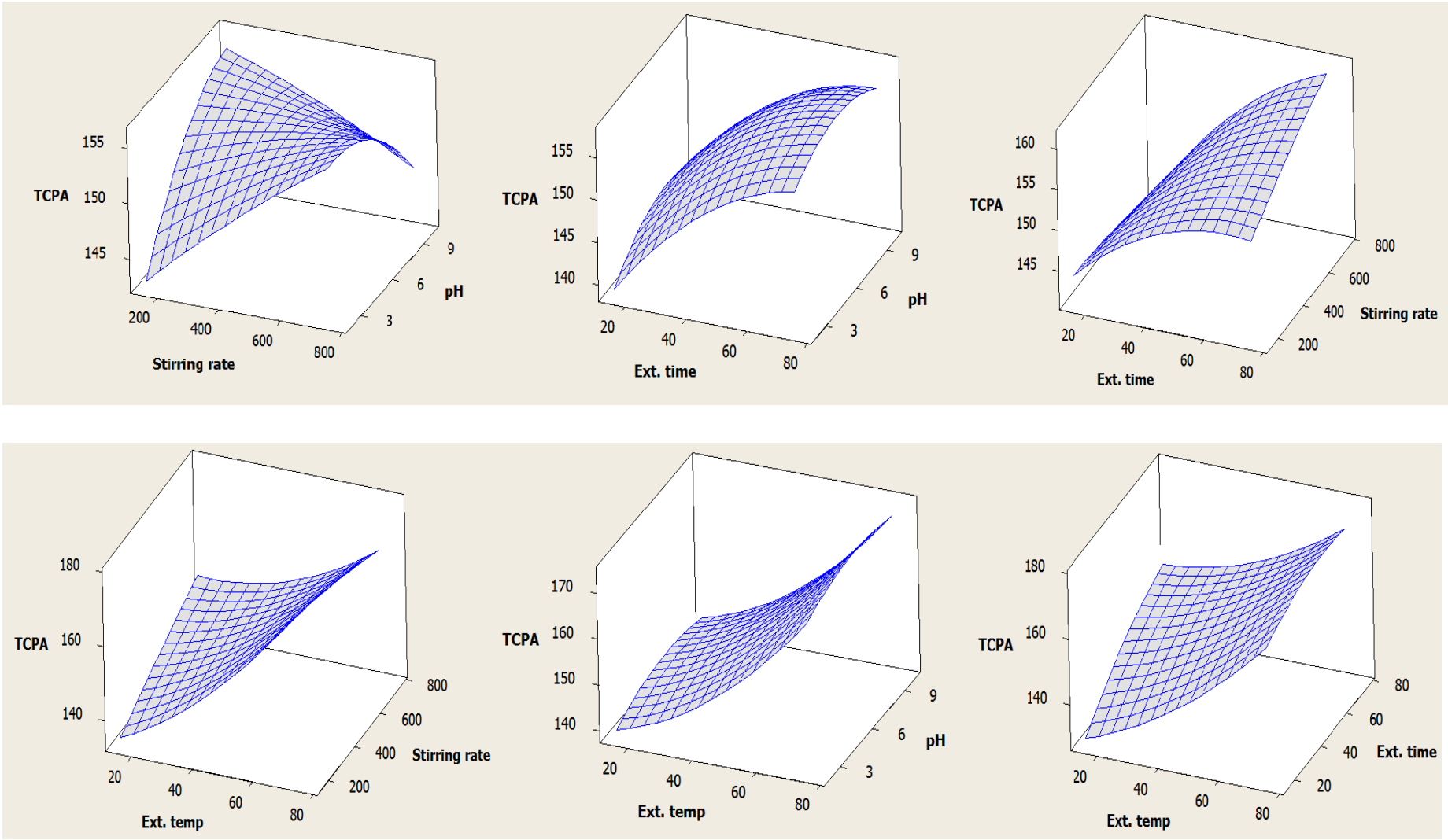

Fig 6: Desirability response surface from CCD

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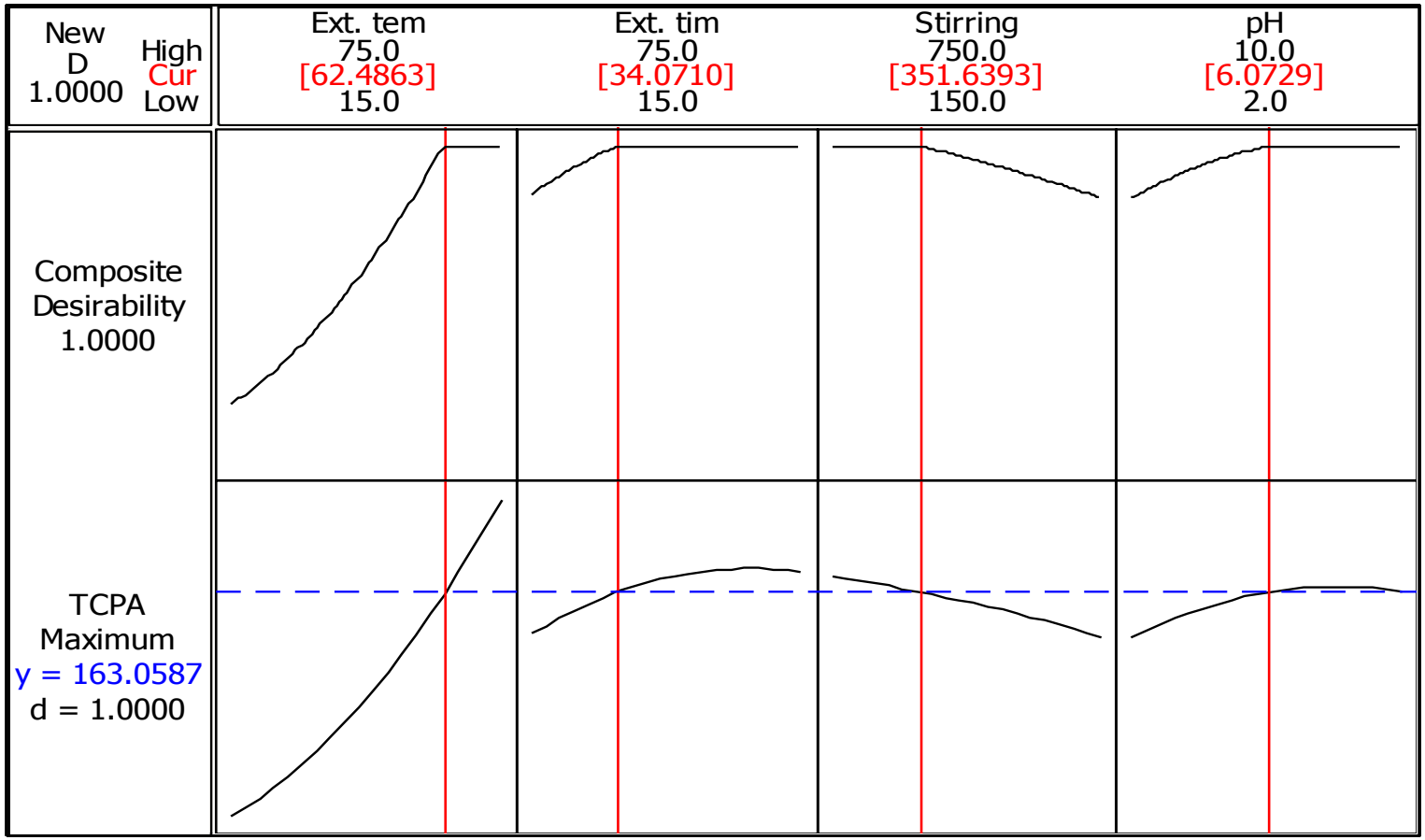

Fig 7: Response optimizer for optimized parameters

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Table 4: Optimized extraction conditions

Factors	Optimized condition
SPME fiber	PDMS
Extraction temperature (^{0}C)	65
Extraction time (min)	35
Salt addition (%, v/v)	10
Stirring rate (rpm)	350
pH	6
Desorption time (min)	7
Desorption temperature (^{0}C)	270

Method Validation

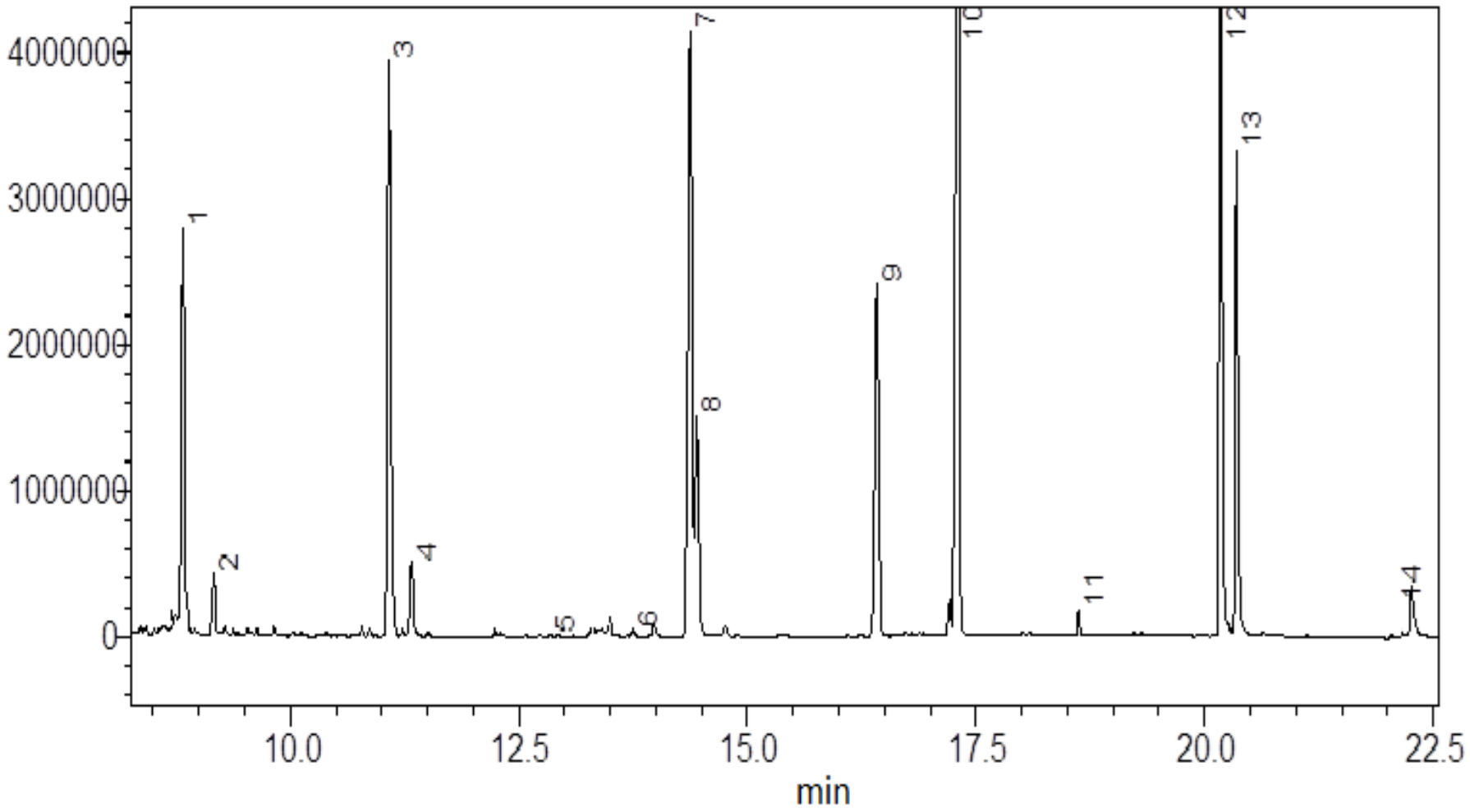

Fig 8: GC-MS Chromatogram of tomato sample spiked at 100 µg/kg

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Method Validation

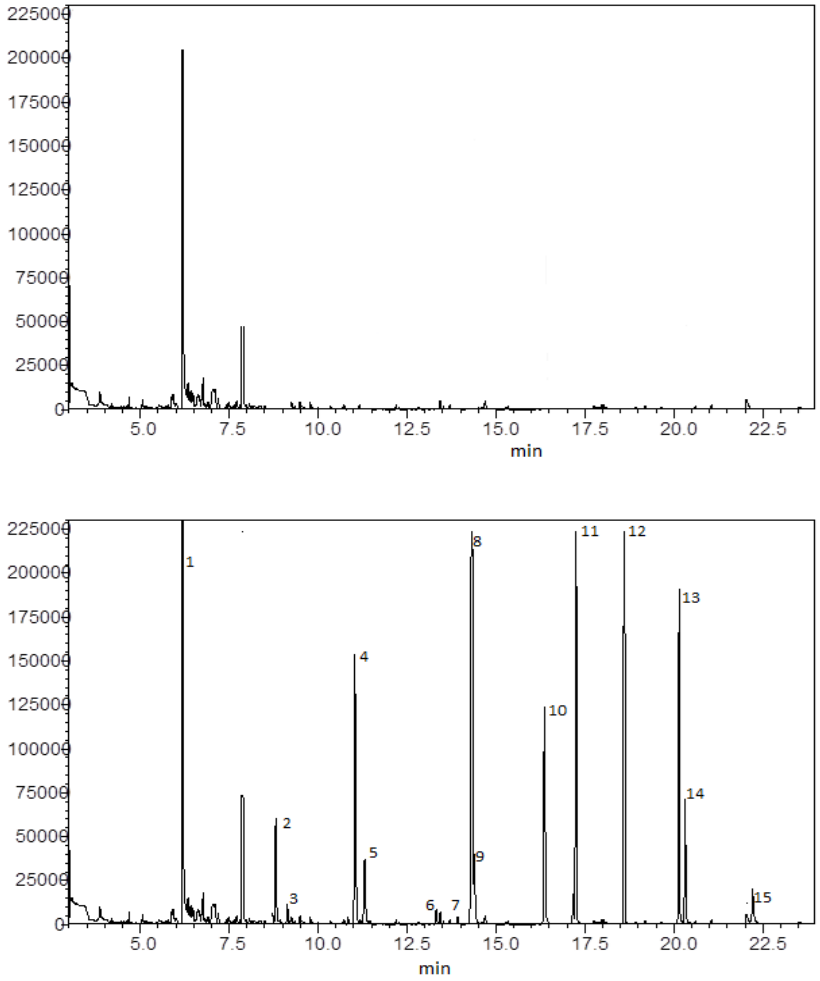

Fig 8: GC-MS Chromatogram of tomato sample spiked at 100 µg/kg

Table 5: Figures of merit of the developed method

S/N	Analytes	LR (µg/kg)	r^2	LOD (µg/kg)	LOQ (µg/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)

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Table 6: Pesticide residues found in samples collected from 10 farms(µg/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 (µg/kg) of the developed HS-SPME method

Pesticides	Ret. Time (min)	Ion m/z	Range (µg/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

Figures of Merit of the Developed Method in Fruits and Vegetable Samples(µg/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
Ethopropop	LOD	1.31	0.23	2.51	1.2	0.35	0.23	0.34
	LOQ	4.36	0.77	8.36	4	1.15	0.77	1.14
	MRL	20	20	20	20	20	20	20
Diazinon	LOD	0.88	0.21	0.51	1.05	0.32	0.21	0.23
	LOQ	2.92	0.68	1.84	3.5	1.05	0.68	0.77
	MRL	10	10	10	10	10	10	10
Chlorothalonil	LOD	2.16	6.94	4.76	0.43	8.33	6.8	0.51
	LOQ	7.21	23.12	15.86	1.44	27.76	22.67	1.84
	MRL	1000	2000	1000	10	1000	1000	10
Parathion-methyl	LOD	0.24	0.62	0.27	0.22	0.5	0.53	0.59
	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(µg/kg)

Pesticide		Apple	Tomato	Pear	Grape	Cucumber	Cabbage	Lettuce
Fenitrothion	LOD	0.53	1.35	0.23	0.2	0.26	0.68	0.2
	LOQ	1.77	4.48	0.88	0.66	0.85	2.25	0.67
	MRL	10	10	10	10	10	10	10
Chlorpyrifos	LOD	3.3	3.71	3.17	2.79	2.96	3.3	3.52
	LOQ	11.01	12.36	10.58	9.29	9.87	11	11.75
	MRL	500	500	500	500	500	500	500
Thiobencarb	LOD	3.48	4.34	3.42	3.19	4.03	3.77	3.19
	LOQ	11.58	14.47	11.4	10.62	13.43	12.57	10.62
	MRL	100	100	100	100	100	100	100
Quinalphos	LOD	2.16	1.94	2.41	2.24	1.94	1.97	2.05
	LOQ	7.37	6.48	8.03	7.47	6.48	6.6	6.83
	MRL	50	50	50	50	50	50	50
Endosulfan I	LOD	2.3	3.91	2.76	3.45	3.25	3.13	2.27
	LOQ	7.67	13.14	9.2	11.5	10.83	10.43	7.57
	MRL	50	50	50	50	50	50	50

Figures of Merit of the Developed Method in Fruits and Vegetable Samples(µg/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 (µg/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

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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

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