

학술상 수상강연

Matrix Enhancement Effect: A Blessing or a Curse for Gas Chromatography?

Jae-Han Shim

심재한

Natural Products Chemistry Laboratory, Chonnam National University, Gwangju, Republic of Korea
전남대학교 농업생명과학대학

The matrix enhancement effect in gas chromatography (GC) has been a problem for the last decade as it results in unexpected high recovery. Most of the efforts, including the use of different types of injectors/matrix simplification procedure, and further clean-up associated with removing this effect was focused on equalizing the response of the standard in the solvent and matrix. However, after eliminating the matrix enhancement effect, the sensitivity of GC remained unchanged. But, GC sensitivity can be increased by utilizing this matrix effect originating from a matrix matched standard. Very few studies have highlighted utilizing the matrix effect but have rather advocated eliminating it. Analyte protectants (3-ethoxy-1, 2-propanediol, gulonolactone and sorbitol) have been introduced as an alternative for GC-mass spectroscopy (GC-MS) (not examined for other GC detectors), as they equalize the response without removing the matrix effect, and, hence, increase sensitivity. Versatile applications of analyte protectants are not observed in practice. The European guidelines recommend use of matrix matched standard calibration for residue measurements. As a result, numerous applications are available for matrix matched standards that compensate for the matrix effect. Moreover, the matrices (among them pepper leaf matrix) can act as a protectant for thermolabile analytes in some cases. A lower detection limit should be achieved from the GC detector to comply with the maximum residue limits. Therefore, the matrix enhancement effect, which is considered a problem, can play an important role in lowering the detection limit by increasing the transfer of analyte from the injection port to the detector.

Key words : gas chromatography, matrix effect, analyte protectants, signal enhancement, thermal protection

Corresponding author : E-mail, jhshim@chonnam.ac.kr; Tel, 82-62-530-2135; Fax, 82-62-530-0219

Matrix enhancement effect: A blessing or a curse for gas chromatography?

Jae-Han Shim
Chonnam National University
Republic of Korea

Contents

Literature review

Case Study 1,2,3 and 4

Conclusions

Q and A

Literature review

Importance of pesticides



Benefits and Drawbacks of pesticides





Quality of foods



Public health problems

Tools for Pesticide residue analysis

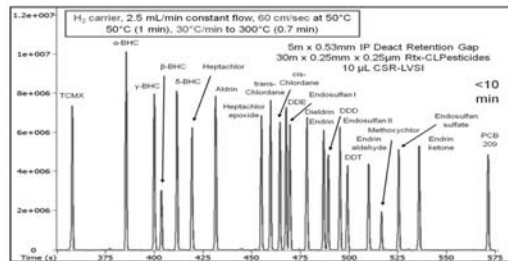


Gas chromatography (GC)



Liquid chromatography (LC)

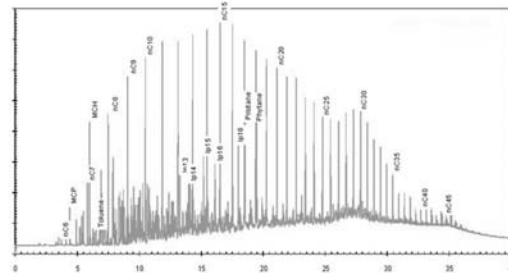
High separation power



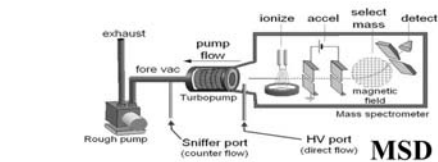
GC column



Low limit of detection



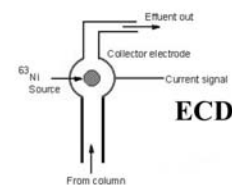
✓ GC, the main working horse in pesticide residue laboratories since late 1960s



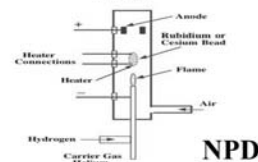
MSD



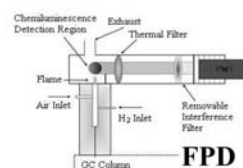
Gas chromatography



ECD

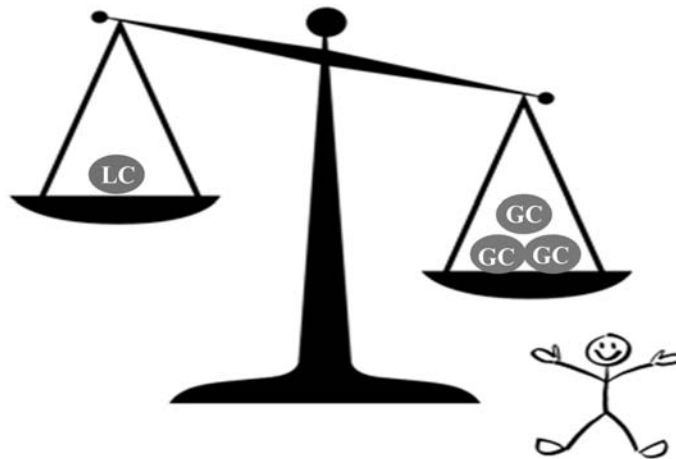


NPD

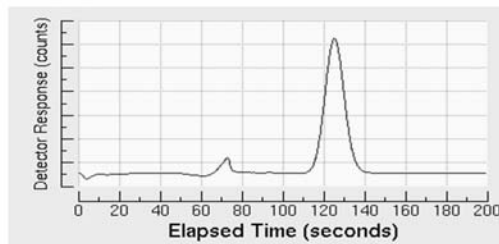


FPD

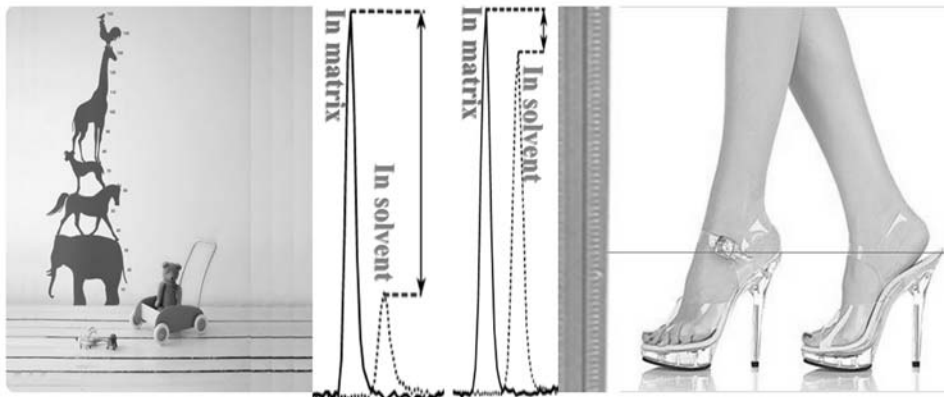
✓ GC methods are still preferred over LC methods



GC

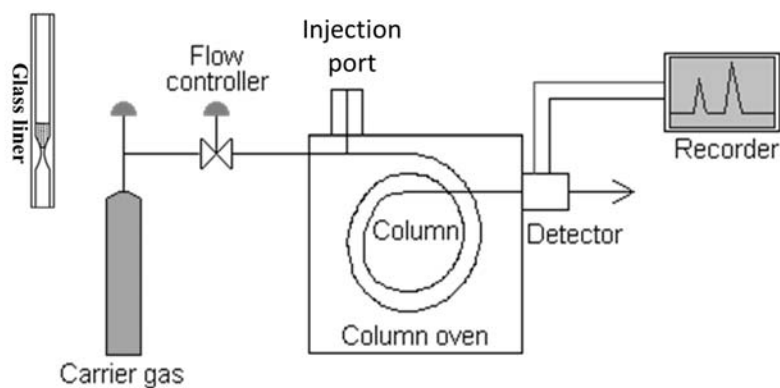


Overestimation

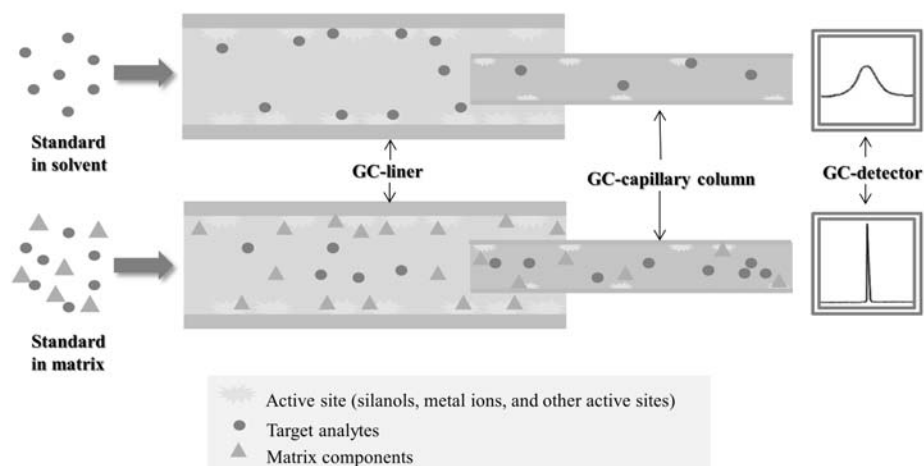


Gas chromatography

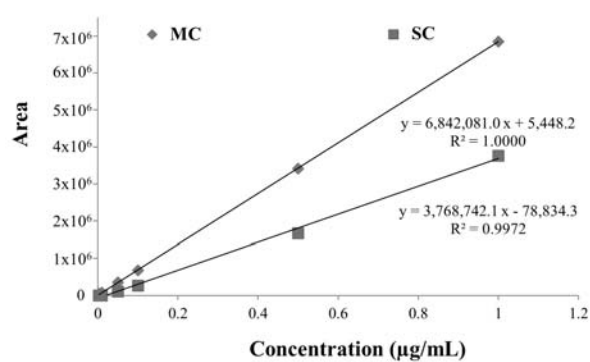
- ✓ In 1993, Erney and his co-workers explained this overestimated recovery and named it “matrix induced response enhancement effect”



Matrix effect



Ref. Erney et. al, 1993



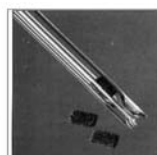
Calibration curve of pymetrozine; standard in chili matrix (MC) and standard in solvent (SC) [unpublished data].

Problems in Gas chromatography

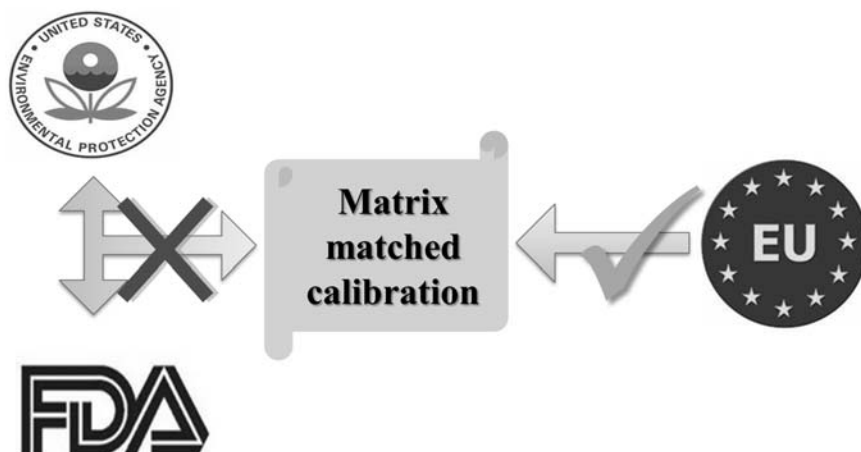
- ✓ GC system (Injection port, capillary column and detector)
 - Not inert
 - Have active sites
- ✓ Causes
 - Less sensitive peak for loss of analytes
 - Peak alteration
 - Poor peak shape & peak tailing
 - Standard decomposition

Existing Solutions

- ✓ Derivatization
- ✓ The use of different injection techniques
 - Pulsed splitless
 - Cold on-column
 - Programmed temperature vaporizer (PTV)
- ✓ A plug of carbofrit inserted in the glass liner
- ✓ Olive oil or corn oil
- ✓ Matrix-matched calibration standards or analyte protectants



Regulations



Use of Single additive

- ✓ Many attempt were taken to compensate matrix enhancement effect
- ✓ Using single additive to equalize the response between the standard in pure solvent and in matrix
- ✓ Six compounds were examined to deactivate the active site and protect the analytes in pure solvent

- I. 1, 2, 3,-Tris (2-cyanoethoxy) propane,
- II. 2. N, N, N', N'-tetrakis (2-hydroxypropane) ethylenediamine
- III. Glycerine,
- IV. poly (ethylene glycol) 200
- V. formic acid
- VI. formamide

Different injection techniques

- ✓ Pulsed splitless, cold on-column or programmed temperature vaporizer (PTV), carbofrit inserted in the glass liner, can diminish matrix effect but not eliminate it
- ✓ Direct sample introduction (DSI), or difficult matrix introduction (DMI), have been also tested, but the total elimination of sample components is not possible

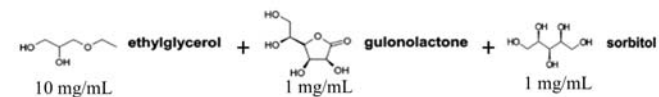
Extensive clean-up

- ✓ Matrix effect was tried to diminish by reducing matrix component through extensive clean-up
- ✓ Different types of SPE cartridges GCB, PSA, and SAX were employed
- ✓ GCB+SAX+PSA was found to reduce matrix enhancement effect more than the other SPE approaches
- ✓ Extensive purification does not allowed due to the different physico-chemical properties, time consuming and laborious

Additive re-introduction

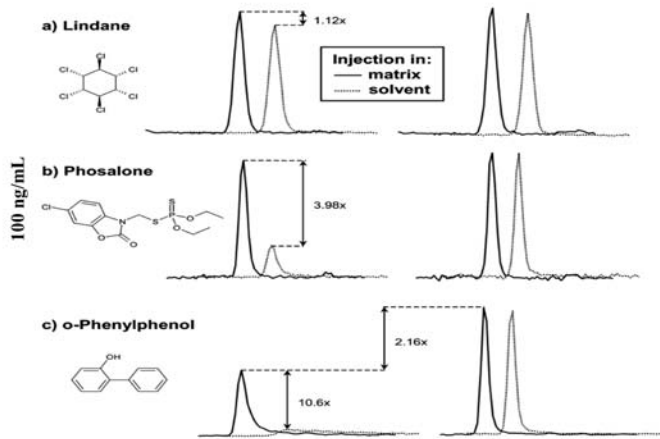
- ✓ Pesticides including (–OH), (R–NH–) (–N=), (–O–CO–NH), (–NH–CO–NH–), (–P=O) are the most affected analytes
- ✓ Various polyol and their derivative, amino acid, carboxylic acid, basic derivative of nitrogen containing heterocyclic group
- ✓ A combination of **ethylglycerol**, **gulonolactone**, and **sorbitol** was found to be the most effective protectant in GC-MS

Analyte protectant



A) without analyte protectants

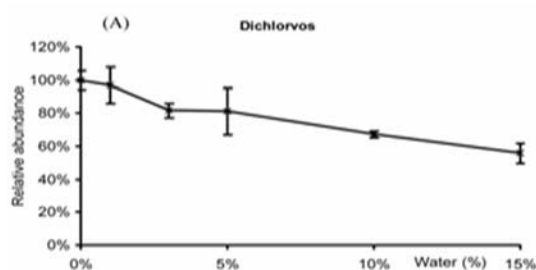
B) with analyte protectants



Ref. Mas'tovska' et. al, 2005

Drawback of analyte protectants

- ✓ Ethyl glycerol (100 mg/mL) dissolved in acetonitrile (MeCN)
- ✓ Sorbitol (10 mg/mL) dissolved in 85:15 (v/v) MeCN/water
- ✓ Gulonolactone (20 mg/mL) dissolved in 80:20 (v/v) MeCN/water



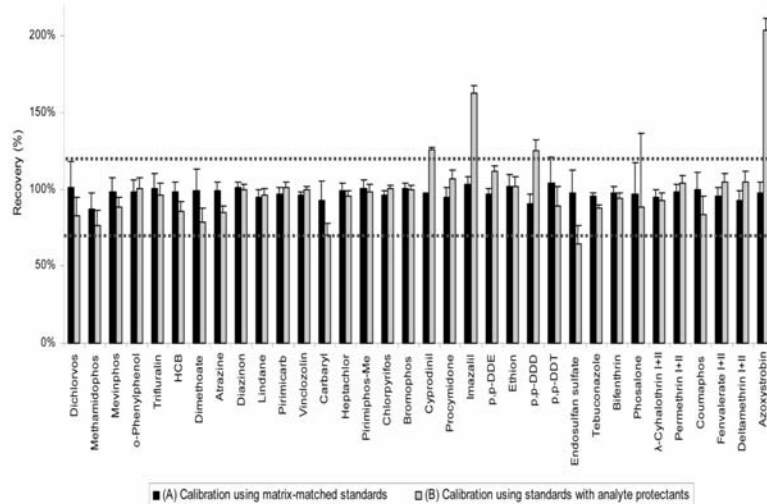
Influence of water content in MeCN on the analyte peak height for dichlorvos

Ref. C. ajka et. al, 2005

Injection of MeCN in GC

- ✓ Poor focusing of chromatographic peaks due to the high polarity of MeCN
- ✓ Limitations on injection volumes due to the high expansion coefficient of MeCN
- ✓ Contamination of the system by matrix co-extractives

Comparison of MMC and analyte protectants

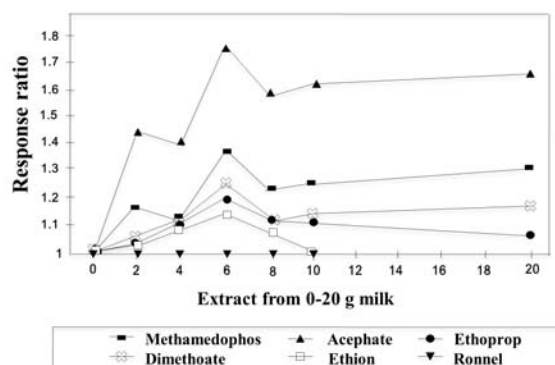


Ref. C[~]ajka et. al, 2005

Matrix matched calibration (MMC)

- ✓ The matrix effect gives larger and higher quality peaks, better to take advantages of this phenomenon rather than eliminating it
- ✓ Matrix itself can consider as analyte protectant if it can perfectly protect the analytes
- ✓ The protection capability varies among matrices and their concentrations

Matrix matched calibration (MMC)



The response ratios in Figure show a general trend of increasing to a maximum when milk weight (extract) was increased from 2 to 6 g, less predictable results between 6 to 10 g, then a shallow change (increase to decrease) between 10 to 20 g.

Ref. Erney et. Al. 1997

MMC for lowering LOD

- ✓ If analytes in solvent are affected by active sites inside the GC system the limit of detection (LOD) becomes high
- ✓ Sample amount in traditional LLE and SPE purification procedure was the early practice to lowering the LOD
- ✓ The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method need lower LOD as it uses 1 g/mL sample
- ✓ In matrix matched calibration if the matrix itself as an analyte protectants, lower the detection limit, overcome overestimation

Objectives

The aim of this study was to investigate the common problems in gas chromatography, utilization of matrix enhancement effect for more sensitive gas chromatography analysis, and introduce pepper leaf matrix as a promising natural analyte protectant for thermolabile analytes

Literature review

Case Study 1,2,3 and 4

Conclusion

Q and A



Case Study 1,2,3 and 4

Problems in Gas chromatography

✓ GC system (Injection port, capillary column and detector)

- Not inert
- Have active sites

✓ Causes

- **Loss of analytes** → Unacceptable recovery percentage
- **Peak alteration** → Difficult to identify and integrate
- **Poor peak shape & peak tailing** → Higher detection limit
- **Standard decomposition** → Impossible to detect

Case 1 Loss of analytes

Single-step modified QuEChERS for determination of chlorothalonil in shallot (*Allium ascalonicum*) using GC- μ ECD and confirmation via mass spectrometry

Rahman *et al.*, 2012; *Biomed. Chromatogr.*, 27(4), 416-421.

Introduction

- ✓ Analyte at lower concentration are more prone to degradation since overestimation observed at lower level of spiking
- ✓ LOD and LOQ were badly affected since the analyte losses during solvent injection
- ✓ Chlorothalonil was unstable to the QuEChERS extraction for using PSA clean-up as it causes pH increment
- ✓ Only matrix matched calibration was the solution for lowering limit of detection avoiding overestimation

Experimental objective

The aim of this study was to modify QuEChERS sample preparation method using matrix matched calibration for determination of chlorothalonil in shallot

Experimental

✓ **Sample**

Shallot

✓ **Standard**

Chlorothalonil

✓ **Reagents**

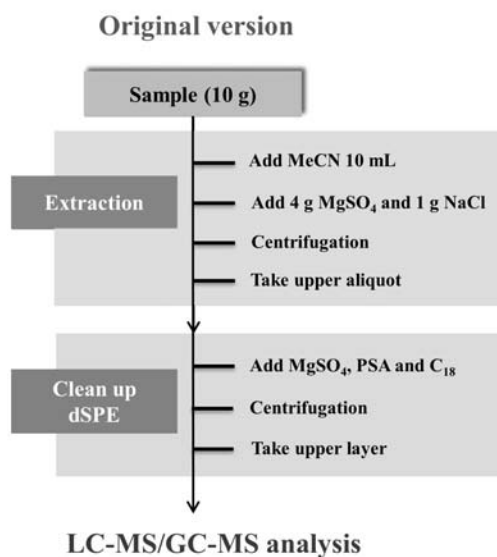
NaCl, MgSO₄ (anhydrous), ethyl acetate (EtOAc)

Instrumental conditions

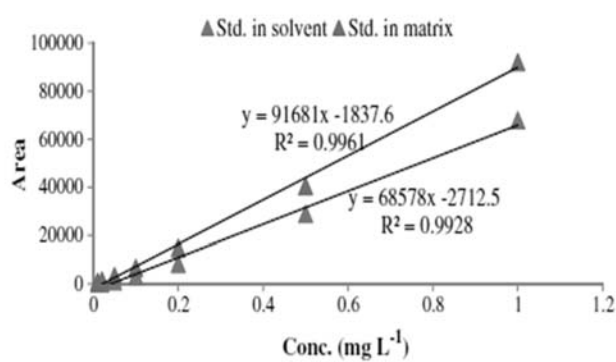
Instrumental conditions of GC- μ ECD for detecting chlorothalonil in shallot

Model	Agilent 7890A equipped with μ ECD		
Column	HP-Ultra 2 (50 m \times 0.32 mm i.d, 0.17 μ m film thickness, Agilent, USA)		
Temperature	Oven		
	Injector	270°C	
	Detector	300°C	
	Carrier gas	N ₂ 1.5 mL/min	Make up gas 30 mL/min
Injection Volume	1 μ L (Split 10:1)		

Sample preparation

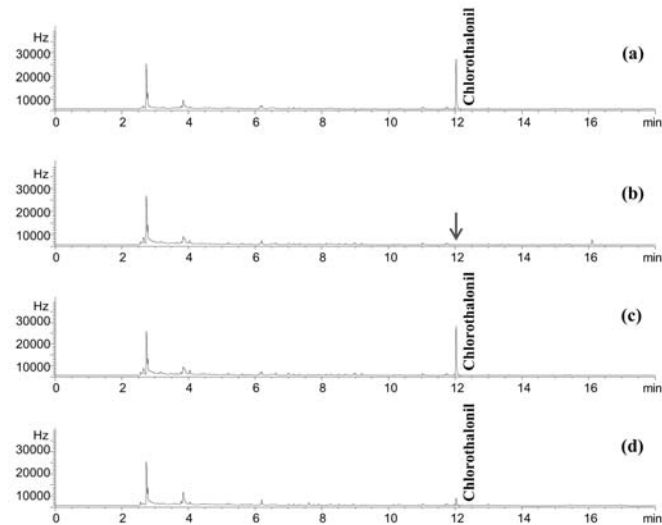


Results and discussion



Calibration curve of standard chlorothalonil prepared in solvent and in shallot extract.

Chromatograms



GC- μ ECD chromatograms of (a) standard chlorothalonil 2 ppm in control matrix; (b) control shallot (c) recovery and (d) field sample

Results and discussion

Recovery, limit of detection and quantification of chlorothalonil in shallot

Compound	LOD (mg L ⁻¹)/ (mg kg ⁻¹)	LOQ (mg L ⁻¹)/ (mg kg ⁻¹)	r^2	Spiked level (mg kg ⁻¹)	Recovery (mean, RSD %)
Chlorothalonil	IDL* 0.003	IQL* 0.01	0.996	0.4	97.2 (1.3)
	MDL** 0.012	MQL** 0.04		2.0	104.9 (2.7)

* IDL-Instrumental detection limit

* IQL-Instrumental quantification limit

** MDL-Method detection limit

** MQL-Method quantification limit

Results and discussion

Residues of chlorothalonil on shallot at various intervals after treatment

Days after treatment	Residues \pm SD (mg kg ⁻¹)	Reduction rate (%)	Half life (days)
0	6.25 \pm 0.41	-	
1	5.98 \pm 0.12	4.32	
2	4.25 \pm 0.28	32.00	
3	2.49 \pm 0.20	60.16	2.8
5	1.88 \pm 0.05	69.92	
7	0.96 \pm 0.07	84.64	
10	0.75 \pm 0.06	88.00	
14	0.18 \pm 0.01	97.12	

Conclusions

QuEChERS drawback for the analysis of chlorothalonil has been successfully overcome after avoiding dSPE clean-up, modifying sample solvent ratio and employing matrix matched calibration for analyte protection

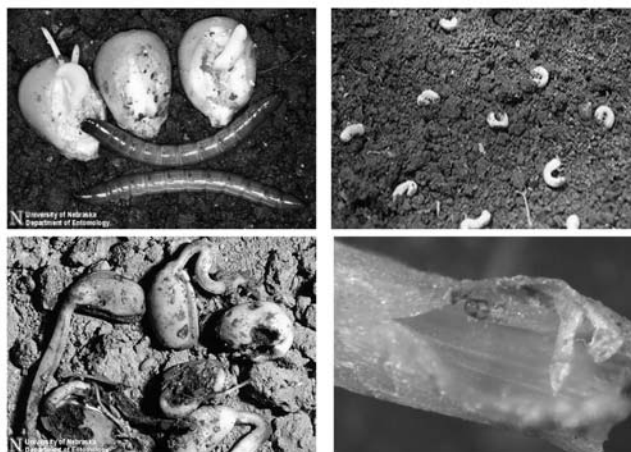
Case 2 Peak alteration

Pepper leaf matrix as a promising analyte protectant prior to the analysis of thermolabile terbufos and its metabolites in pepper using GC-FPD

Rahman *et al.*, 2012; *Food Chemistry*, 133, 604-610.

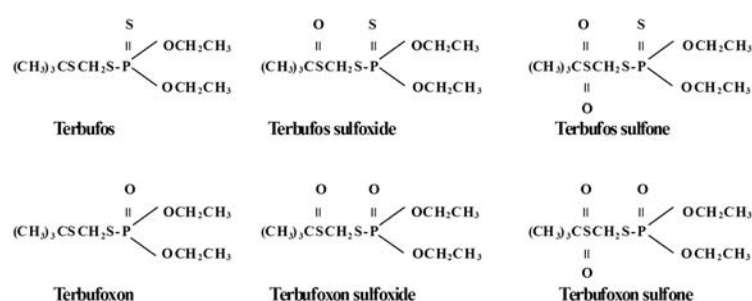
Introduction

- ✓ Terbufos, an organophosphate insecticide and nematicide, was used to control wireworms, seedcorn maggots, white grubs, corn rootworm larvae, and other pests

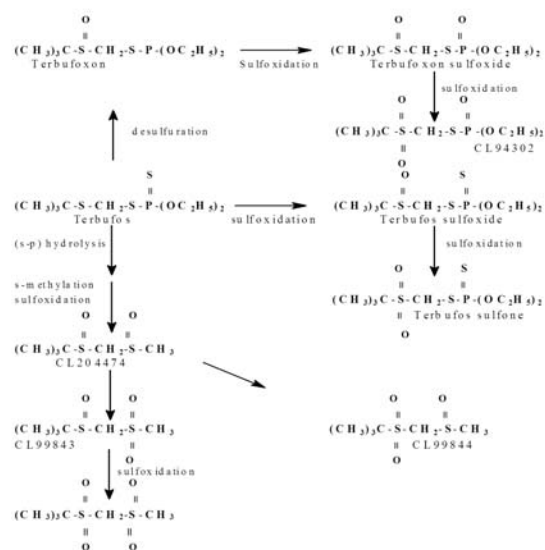


Introduction

- ✓ Oxidative metabolites in plants (P = S and P = O sulfoxides and sulfones) must be taken into account in residue analysis, which are similarly toxic but more mobile and persistent than the parent compound



Terbufos and its five toxic metabolite



Proposed metabolic pathway in plants

Experimental objectives

The aim of this study was to develop an analytical method to analyze terbufos and its metabolites individually in pepper and pepper leaf samples

Experimental

✓ Samples

Pepper and pepper leaf

✓ Standards

Group 1

Terbufos
Terbufoxon
Terbufos sulfone

Group 2

Terbufoxon sulfoxide
Terbufos sulfoxide
Terbufoxon sulfone

✓ Reagents

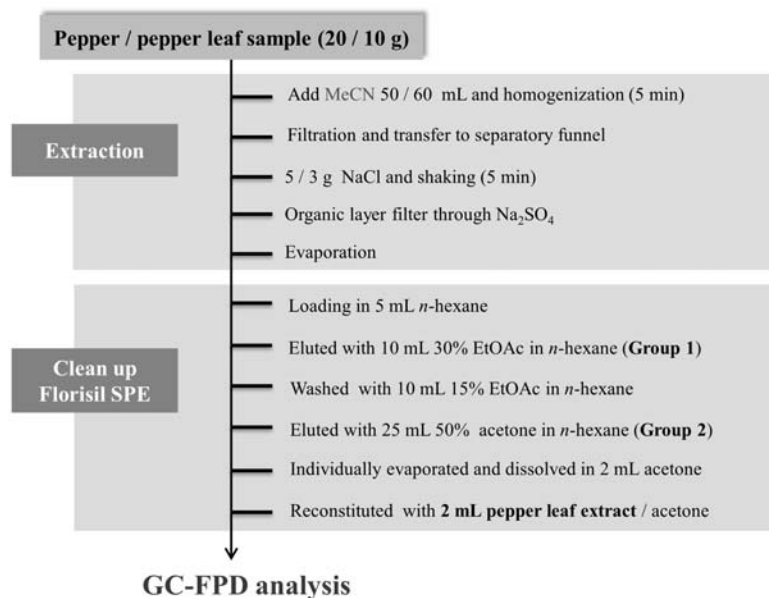
NaCl, Na₂SO₄ (anhydrous)
acetonitrile, acetone, EtOAc, *n*-hexane

Instrumental conditions

Instrumental conditions of GC-FPD for detecting terbufos and its metabolites

Model	Shimadzu GC-17A- Flame photometric detector with P-filter	
Column	HP-5 capillary column (30 m×0.53 mm I.D.×1.5 µm film thickness)	
Temperature		
	Injector	290°C
	Detector	180 °C (head) & 290°C (base)
	Carrier gas	N ₂ 2 mL/min
Injection Volume	2 and 5 µl	

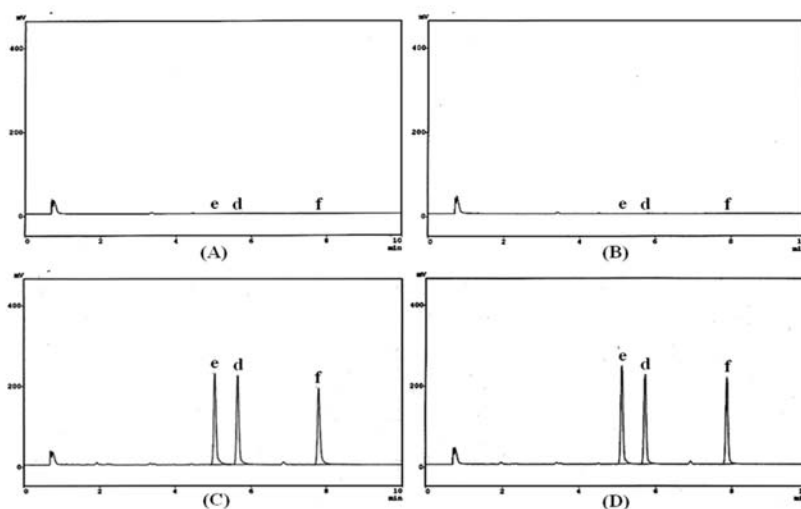
Sample preparation



Results and discussion

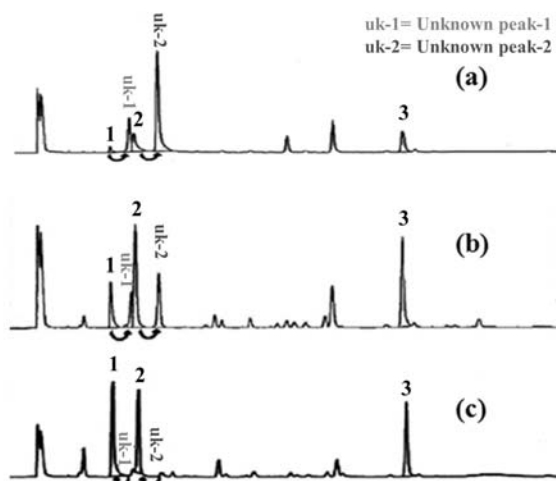
- ✓ All of the analytes were comparatively stable except terbufos sulfoxide and terbufoxon sulfoxide
- ✓ Terbufos sulfoxide and terbufoxon sulfoxide were highly thermolabile, and were readily altered inside the GC system
- ✓ Pepper matrix, analyte protectant, and carbofrit inlet liner failed to completely protect these compound
- ✓ Only pepper leaf matrix could protect the metabolites against alteration

Chromatograms



GC-FPD Chromatograms of Group-1, Blank pepper matrix (A), Blank pepper leaf matrix (B); group-1 in pepper matrix (C) and in pepper leaf matrix (D); d, terbufos; e, terbufoxon; f, terbufos sulfone.

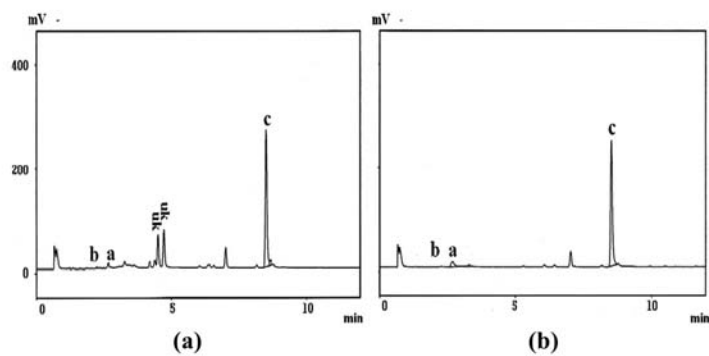
Results and discussion



1: terbufoxon sulfoxide, 2: terbufos sulfoxide, 3: terbufoxon sulfone

Terbufos metabolites mixture 5 ppm a) in solvent; b) in pepper matrix; c) in pepper leaf matrix.

Results and discussion



b=terbufoxon sulfoxide, a= terbufos sulfoxide, c= terbufoxon sulfone

Terbufos metabolites mixture 10 ppm using a) analyte protectant; b) carbofrit inlet liner.

Results and discussion

Correlation coefficient (r^2), limit of detection (LOD), limit of quantification (LOQ), and recovery of the six analytes in pepper and pepper leaf samples

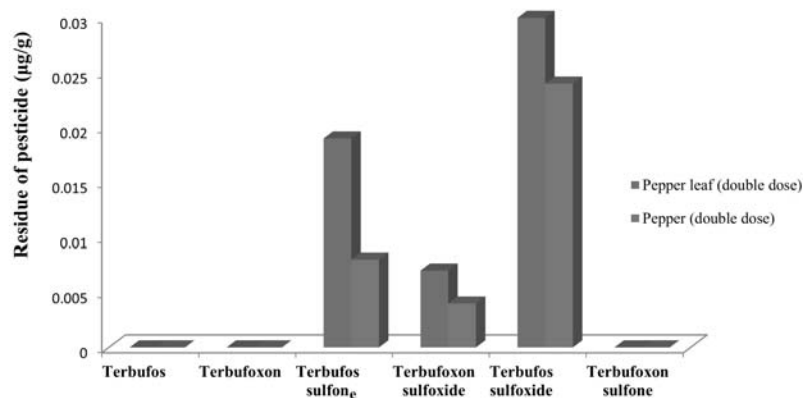
Compound	Pepper/pepper leaf					
	r^2	LOD (mg/kg)	LOQ (mg/kg)	MRL ^a (mg/kg)	Recovery (%; n = 3)	
					LOD × 10 mg/kg	LOD × 50 mg/kg
Terbufos	0.997/0.997	0.001/0.002	0.003/0.007	0.05/0.05 ^b	71.8 (3.5)/73.8 (3.0) ^c	90.9 (1.2)/84.2 (2.8)
Terbufos sulfoxide	0.994/0.996	0.002/0.004	0.007/0.013	-/-	87.9 (5.9)/114.1 (2.9)	79.5 (10.8)/74.2 (2.5)
Terbufos sulfone	0.997/0.992	0.001/0.002	0.003/0.007	-/-	73.0 (3.1)/73.9 (1.0)	91.5 (4.7)/91.7 (4.0)
Terbufoxon	0.998/0.999	0.001/0.002	0.003/0.007	-/-	69.1 (3.1)/72.2 (3.8)	75.0 (1.6)/74.5 (5.9)
Terbufoxon sulfoxide	0.997/0.998	0.0005/0.001	0.002/0.003	-/-	86.5 (11.1)/114.5 (2.5)	87.6 (5.0)/78.2 (2.5)
Terbufoxon sulfone	0.990/0.999	0.001/0.002	0.003/0.007	-/-	90.3 (9.2)/74.3 (3.6)	96.5 (8.7)/96.0 (4.4)

^a Residue definition of terbufos; the sum of terbufos, its oxygen analogue and their sulfoxides and sulfones, expressed as terbufos for compliance with MRLs.

^b MRL of terbufos in Chinese cabbage (KFDA, 2011).

^c Relative standard deviation.

Results and discussion



Residues of terbufos, terbufoxon, terbufos sulfone, terbufoxon sulfoxide, terbufos sulfoxide, and terbufoxone sulfone in pepper and pepper leaf for double dose application.

Conclusions

Pepper leaf matrix could protect highly unstable compound terbufos sulfoxide and terbufoxon sulfoxide where only pepper matrix, chemical analyte protectant and carbofrit inlet liner were failed to protect them inside the GC system

Case 3 Poor peak shape & peak tailing

Analysis of kresoxim-methyl and its thermolabile metabolites in Korean plum: An application of pepper leaf matrix as a protectant for GC amenable metabolites

Rahman *et al.*, 2013; *J. Sep. Sci.*, 36, 203-211.

Determination of kresoxim-methyl and its thermolabile metabolites in pear utilizing pepper leaf matrix as a protectant using gas chromatography

Rahman *et al.*, 2013; *Journal of Advanced Research*, (In press)

Introduction

- ✓ Kresoxim-methyl a recently developed strobilurin fungicide, is used for the control of powdery mildew and scab
- ✓ For risk assessment, residue definition was proposed as sum of kresoxim-methyl and its metabolites, BF 490-2 and BF 490-9
- ✓ Very few unpublished complex laboratory method was reported for metabolites analysis using HPLC-UVD
- ✓ An efficient analytical method for the determination of kresoxim-methyl and its metabolites was still lacking

Experimental objectives

The aim of the present study was to develop a gas chromatography method for kresoxim-methyl and its thermolabile metabolites using pepper leaf matrix as an analyte protectant to estimate the residual levels in Korean plum and pear

Experimental

Reagents and Materials

➤ Samples

- ❖ Korean plum and pear

➤ Standards

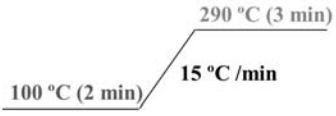
- ❖ Kresoxim-methyl, BF 490-2 and BF 490-9

➤ Reagents

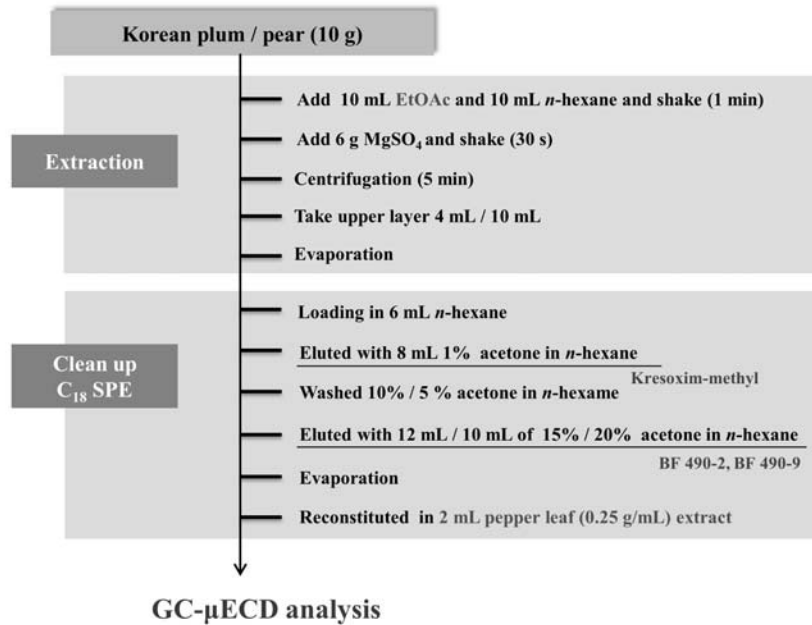
- ❖ MgSO_4 (anhydrous), acetone, ethyl acetate (EtOAc) and *n*-hexane

Instrumental conditions

Instrumental conditions of GC- μ ECD for detecting kresoxim-methyl and metabolites

Model	Agilent 7890A equipped with μ ECD			
Column	HP-5 capillary column (30 m×0.53 mm I.D.×1.5 μ m film, thickness)			
Temperature	Oven			
	Injector	270°C		
	Detector	300°C		
Carrier gas	N_2	2 mL	Make up	60 mL
Injection Volume	1 μ l			

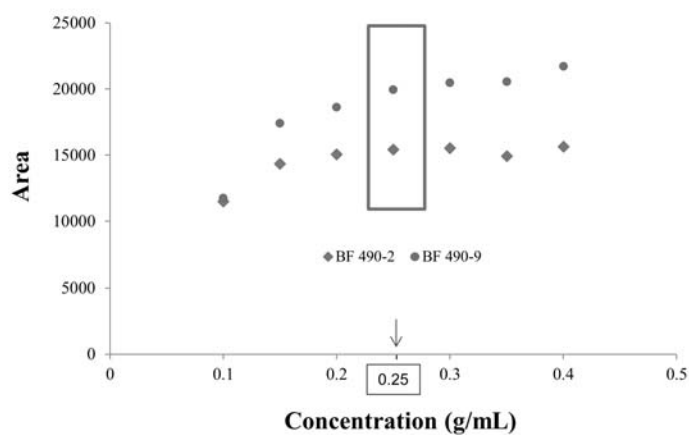
Sample preparation



How to make pepper leaf matrix



Optimization of pepper leaf matrix for kresoxim-methyl metabolites

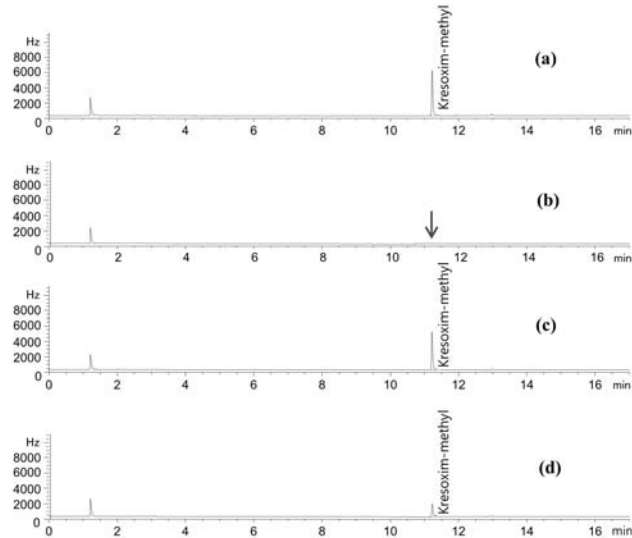


Responses of BF490-2 and BF490-9 with different concentration of pepper leaf matrix.

Results and discussion

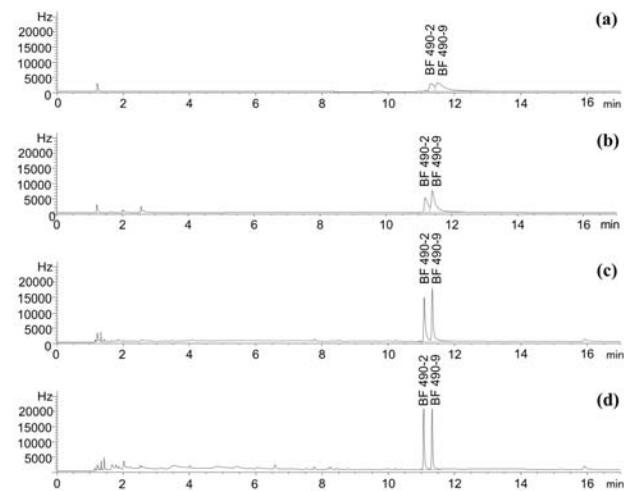
- ✓ Two metabolites of kresoxim-methyl BF 490-2 and BF 490-9 showed low response or poor peak shape in solvent
- ✓ Purified extract of Korean plum and pear was unable to completely protect the thermolabile metabolites
- ✓ Unclean plum and pear extract led to dirty chromatograms with lots of interferences
- ✓ Optimized amount of pepper leaf matrix in combination with purified plum and pear matrix provided perfect protection

Results and discussion



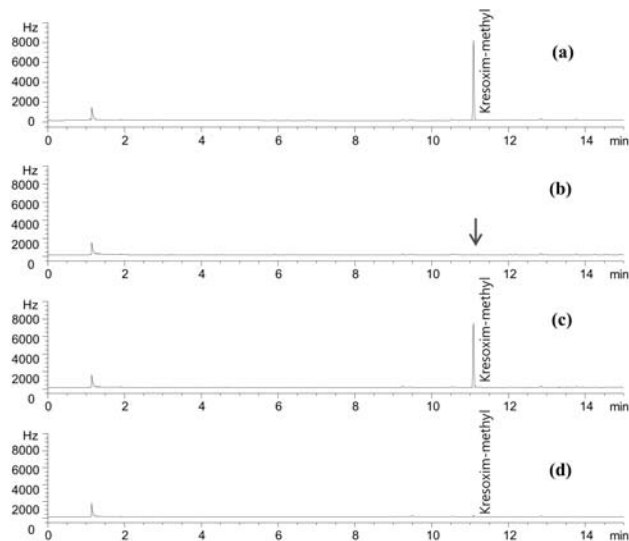
Chromatograms of kresoxim-methyl (a) standard 2.5 mg/kg in matrix (b), blank sample, (c) recovery, and (d) field incurred plum sample.

Results and discussion



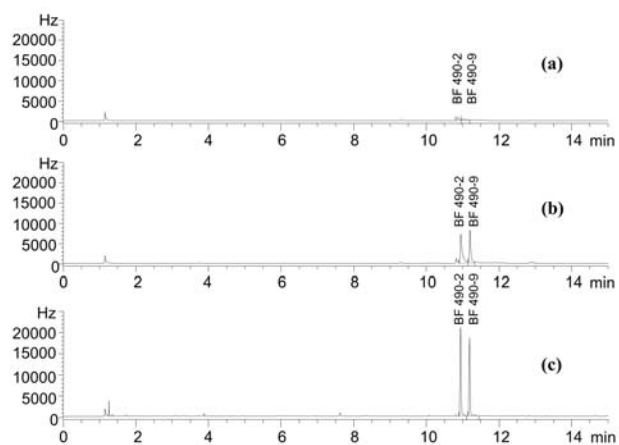
Kresoxim-methyl metabolites mixture 5 ppm a) in solvent; b) in purified plum matrix; c) in pepper leaf matrix; d) in plum+pepper leaf matrix.

Results and discussion



Chromatograms of kresoxim-methyl (a) standard 2.5 mg/kg in matrix, (b) blank sample, (c) recovery equivalent to 2.5 mg/kg, and (d) field incurred pear sample.

Results and discussion



GC- μ ECD chromatograms of BF 490-2 and BF 490-9 5 ppm std. mixture (a) in solvent; (b) in pear matrix and (c) in pear and pepper leaf matrix.

Results and discussion

LOD and LOQ, linear calibration curve, coefficients of determination (r^2), recoveries, and RSD of kresoxim-methyl, BF 490-2, and BF 490-9 in Korean plum

Compound	LOD mg/kg	LOQ mg/kg	Calibration curve ^{a)}	r^2	Recovery% (10 times)	RSD% $n = 3$	Recovery% (50 times)	RSD% $n = 3$
Kresoxim-methyl	0.015	0.05	$y = 5657x - 43.89$	0.999	88.0	3.30	101.4	0.95
BF 490-2	0.015	0.05	$y = 9579x - 327.5$	0.999	74.7	2.99	74.3	2.24
BF 490-9	0.015	0.05	$y = 8789x - 296.1$	0.999	88.6	4.42	81.5	0.67

a) y is the peak area in μ ECD chromatogram; x is the concentration (mg/kg).

Results and discussion

LOD and LOQ, linear calibration curve, coefficients of determination (r^2), recoveries, and RSD of kresoxim-methyl, BF 490-2, and BF 490-9 in pear

Compound	r^2	LOD mg/kg	LOQ mg/kg	Recovery (Mean, RSD %)	
				0.2 mg.kg ⁻¹	1 mg.kg ⁻¹
Kresoxim methyl	0.999	0.006	0.02	92.5 (1.9)	92.4 (2.3)
BF 490-2	0.995	0.02	0.065	93.3 (2.0)	88.7 (1.1)
BF 490-9	0.992	0.02	0.065	97.9 (0.6)	85.6 (0.9)

Conclusions

The thermolabile metabolites of the present study showed low response or poor peak shape in solvent, plum matrix or in pear matrix and were analyzed using pepper leaf matrix. Therefore, pepper leaf matrix proved to be a promising analyte protectant for thermolabile metabolites

Case 4 Standard decomposition

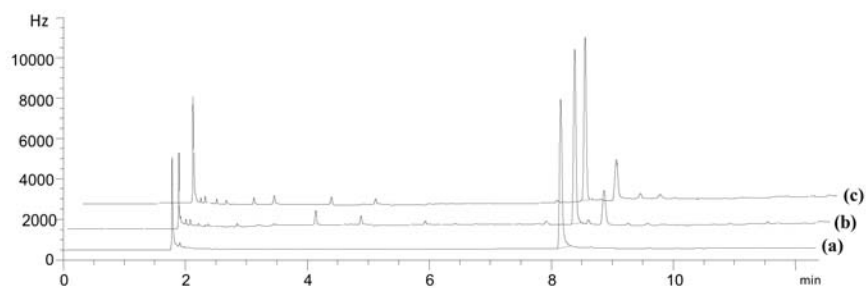
Determination of alachlor residues in pepper and pepper leaf using gas chromatography and confirmed via mass spectrometry with matrix protection

Rahman *et al.*, 2013; *Biomed. Chromatogr.*, (In Press).

Introduction

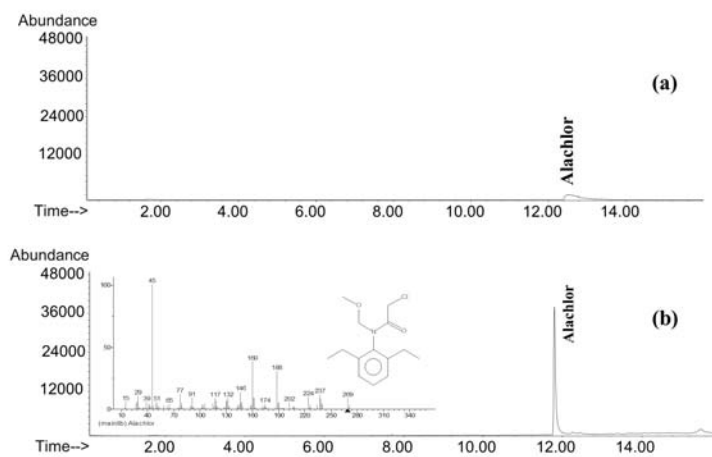
- ✓ Alachlor when analyzed using the HP-Ultra 2 capillary column coupled with GC- μ ECD, it provided sharp and sensitive peak
- ✓ When attempt to confirmed via GC-MS installed with HP-5MS at cleaned condition, peak was disappeared
- ✓ Liner size, design, and column dimensions can play important roles in decreasing the number of active sites
- ✓ Finally, pepper leaf matrix was added as an analyte protectant and confirmed alachlor in field sample via spectrometry

Results and discussion



GC- μ ECD Chromatograms of the standard solutions at the concentrations of 2 mg/L in (a) pure solvent; (b) pepper matrix extract; (c) pepper leaf matrix extract.

Results and discussion



GC-MS chromatograms of alachlor 5 ppm in (a) solvent; (b) pepper leaf matrix.

Literature review

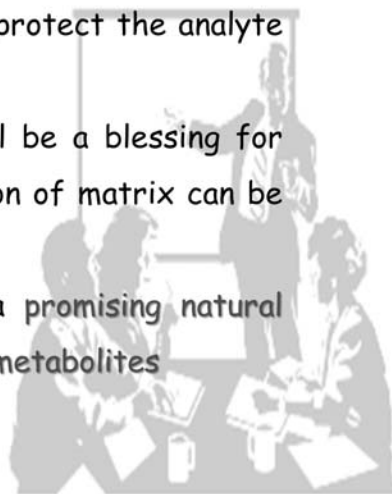
Case Study 1,2,3 and 4

Conclusions

Q and A

Conclusions

- ✓ A sharp, narrow, and sensitive peak is a pre-condition for a sensitive GC analysis
- ✓ An analyte protectant is needed to protect the analyte from any types of peak distortion
- ✓ The matrix enhancement effect will be a blessing for GC only when types and concentration of matrix can be optimized for a particular pesticide
- ✓ Pepper leaf matrix Proved to be a promising natural analyte protectant for thermolabile metabolites



Acknowledgement

