# **TABLE OF CONTENTS**

	Page
TITLE PAGE	i
APPROVAL PAGE	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
TABLE OF CONTENTS	X
LIST OF TABLES	XV
LIST OF ILLUSTRATIONS	xvii
ABBREVATIONS AND SYMBOLS	xix

#### CHAPTER I: INTRODUCTION

1.1 Vetiver		
1.2 Extraction methods of essential oils		
1.2.1 Simultaneous distillation and extraction	8	
1.2.2 Supercritical fluid extraction	9	
1.2.2.1 Supercritical fluid	9	
1.2.2.2 Application of SFE in the essential oils analysis	13	
1.2.3 Microwave-assisted extraction	15	
1.2.4 Soxhlet extraction	18	

1.2.5 Solid phase microextraction	20		
1.3 Gas chromatography for the analysis of essential oils			
1.4 Hyphenated and multidimensional analysis of essential oils			
1.5 Comprehensive two-dimensional gas chromatography (GC×GC)			
1.5.1 Operation of the modulation system	35		
1.5.2 Data processing	36		
1.5.3 Comprehensive gas chromatography for essential oil analysis	36		
1.6 Gas chromatography-mass spectrometry (GC-MS)			
1.7 Aims and scope of this research			

#### CHAPTER II: EXPERIMENTAL

2.1 Study on highly volatile constituents of Vetiveria zizanioides		
roots grown under different cultivation conditions		
2.1.1 Apparatus and chemicals	44	
2.1.1.1 Apparatus	44	
2.1.1.2 Chemicals	46	
<ul><li>2.1.2 Materials</li><li>2.1.3 Extraction of vetiver root essential oils</li></ul>		
		2.1.4 Analysis of volatile constituents in vetiver root essential oils
2.1.4.1 GC×GC analysis	49	
2.1.4.2 SPME-GCΔGC analysis	50	

Page

	Page
-SPME conditioning	50
-Sample preparation and SPME sampling	51
2.1.4.3 SPME-GC-MS analysis	51
2.1.5 Method for calculation of the Kovát retention indices	52
2.2 Comparative study on volatile constituents of Thai vetiver root	
essential oils subjected to different extraction methods and	
analyzed by comprehensive two-dimensional gas	
chromatography-mass spectrometry	54
2.2.1 Apparatus and chemicals	54
2.2.1.1 Apparatus	54
2.2.1.2 Chemicals	57
2.2.2 Materials	57
2.2.3 Extraction procedures	58
2.2.3.1 Simultaneous distillation and extraction (SDE)	58
2.2.3.2 Supercritical fluid extraction (SFE)	58
2.2.3.3 Microwave-assisted extraction (MAE)	59
2.2.3.4 Soxhlet extraction (SE)	60
2.2.4 Investigation of volatile constituents in vetiver essential oils	
obtained from different extraction methods	61
2.2.4.1 GC-MS analysis	61

	Page	
2.2.4.2 GC $\Delta$ GC-FID and GC $\Delta$ GC-qMS analysis	61	
CHAPTER III: RESULTS AND DISCUSSION		
3.1 Highly volatile constituents of Vetiveria zizanioides roots grown		
under different cultivation conditions	64	
3.1.1 Extraction by Likens-Nickerson simultaneous distillation		
and extraction (SDE) apparatus	64	
3.1.2 GC $\Delta$ GC and SPME-GC $\Delta$ GC analysis	65	
3.1.3 SPME-GC-MS analysis	68	
3.2 Volatile constituents of Thai vetiver root essential oils obtained		
by different extraction methods and analyzed by comprehensive		
two-dimensional gas chromatography-mass spectrometry	77	
3.2.1 Comparison of extraction techniques	77	
3.2.2 Selection of GCAGC-FID and GCAGC-qMS		
column set and conditions	80	
3.2.3 Separation and identification of volatile components		
in vetiver root oils by GC-MS and GC×GC-qMS	83	
3.2.4 Quantitative analysis using GC $\Delta$ GC-FID	122	
CHAPTER IV: CONCLUSIONS	142	

	Page
REFERENCES	144
VITA	157

### LIST OF TABLES

Table	Page
1.1 Critical property of various solvents	10
1.2 Commercially available SPME coatings	22
2.1 Three systems of gas chromatographic instrument in first experiment	45
2.2 Three systems of gas chromatographic instrument in second experiment	56
2.3 The extraction conditions of SFE	59
2.4 Extraction conditions used in MAE extraction for three solvents	60
2.5 GC $\Delta$ GC column sets and temperature programs	63
3.1 Structural assignment and relative peak area percent of the volatile	
components in essential oils obtained from root of vertiver grass	
cultivated in three different systems	71
3.2 Percentage yield of vetiver root oil obtained by different	
extraction methods	78
3.3 Structural assignment and electron impact (EI) mass spectra	
of vetiver root essential oils obtained from all extraction methods	
and analyzed by GC-MS	91

Table	Page		
3.4 Structural assignment and electron impact (EI) mass spectra			
of vetiver root essential oils obtained from all extraction methods			
and analyzed by GC×GC-qMS	108		
3.5 Relative contents of the identified component in Thai vetiver			
oils obtained from different extraction methods using GC×GC-FID	123		
3.6 Average relative contents of the identified component in all			
samples with its variation and total numbers of time detected in			
GC and GC×GC	133		

### LIST OF ILLUSTRATIONS

Figu	Figure		
1.1	Carbon dioxide pressure-temperature phase diagram	12	
1.2	2 Carbon dioxide density-pressure phase diagram		
1.3	MDGC heartcut concept	29	
1.4	Schematic diagram of MDGC instrument	30	
1.5	Schematic diagram of GC×GC system utilizing a cryogenic modulator	33	
2.1	Hydroponic cultivation	47	
2.2	Likens-Nickerson apparatus	48	
3.1	The contour plots of volatile component profiles of vetiver essential		
	oils obtained from normal soil cultivation using A. SPME-GC $\Delta$ GC and		
	B. GC∆GC	67	
3.2	SPME-GC-MS chromatograms of vetiver essential oils obtained		
	from root cultivated in three different systems	70	
3.3	Veiver root extracts obtained from three different extraction methods	79	
3.4	GCAGC-FID contour plots obtained by the three column sets	82	

Figu	re	Page
3.5	GC-MS chromatograms of vetiver root essential oils	86
3.6	GC×GC-qMS chromatograms of all vetiver root extracts	102
3.7	GC×GC contour plot of vetiver root essential oil obtained	
	from SDE method showing peak assignment	107
3.8	The relation between number of times detected and % relative	
	content of some components in GC plot	140
3.9	The relation between number of times detected and % relative	
	content of some components in GC×GC plot	140
3.10	The relation between number of times detected and average	
	% relative content of all identified components in GC plot	141

3.11 The relation between number of times detected and average% relative content of all identified components in GC×GC plot141

## **ABBREVIATIONS AND SYMBOLS**

°C	=	degree celsius
μg	=	microgram
<sup>13</sup> C-NMR	=	carbon thirteen nuclear magnetic resonance
<sup>1</sup> H-NMR	=	proton nuclear magnetic resonance
amu	=	atomic mass unit
atm	=	atmosphere
A.C.	=	applied controller
C <sub>14</sub>	=	tetradecyl
C <sub>19</sub>	=	nonadecyl
C <sub>22</sub>	=	dososyl
C <sub>29</sub>	=	nonasosyl
C <sub>8</sub>	=	octyl
CIP	=	carbonyl iron powder
CW	=	carbowax
D	=	dimension
DMHF	=	dimethyl-4-hydroxy-(2H)-furan-3-one
DVB	=	divinylbenzene
e.g.	=	exempli gratia
etc.	=	et cetera
eV	=	electron voltage

FID	=	flame ionization detector
g/cm <sup>3</sup>	=	gram per cubic centimeter
g/mol	=	gram per mole
GC	=	gas chromatography
GC×GC	=	comprehensive two-dimensional gas chromatography
h	=	hour
HD	=	hydrodistillation
HPLC	=	high performance liquid chromatography
HS	=	headspace
Hz	=	hertz
I.D.	=	diameter
$I_x$	=	Kovát retention indices
I <sub>x</sub> K	=	Kovát retention indices kelvin
K	=	kelvin
K kg/m <sup>3</sup>	=	kelvin kilogram per cubic meter
K kg/m <sup>3</sup> LMCS	=	kelvin kilogram per cubic meter longitudinally modulated cryogenic system
K kg/m <sup>3</sup> LMCS LRI	= = =	kelvin kilogram per cubic meter longitudinally modulated cryogenic system Linear retention indices
K kg/m <sup>3</sup> LMCS LRI m/z	= = =	kelvin kilogram per cubic meter longitudinally modulated cryogenic system Linear retention indices mass to charge ratio
K kg/m <sup>3</sup> LMCS LRI m/z MAD	-	kelvin kilogram per cubic meter longitudinally modulated cryogenic system Linear retention indices mass to charge ratio microwave accelerated distillation
K kg/m <sup>3</sup> LMCS LRI m/z MAD MAE		kelvin kilogram per cubic meter longitudinally modulated cryogenic system Linear retention indices mass to charge ratio microwave accelerated distillation microwave-assisted extraction

MDGC	=	multidimensional gas chromatography
min	=	minute
mL	=	microliter
mm	=	millimeter
MPa	=	megapascal
MS	=	mass spectrometry
Р	=	pressure
PDMS	=	polydimethylsiloxane
PHWE	=	pressurized hot water extraction
psi	=	pound per square inch
q	=	quadrupole
RA	=	relative abundance
RSD	=	relative standard deviation
S	=	second
SC	=	supercritical
SCP	=	single component peak
SCWE	=	supercritical water extraction
SD	=	standard deviation
SDE	=	simultaneous distillation and extraction
SE	=	soxhlet extraction
SF	=	supercritical fluid
SFE	=	supercritical fluid extraction

SFME	=	solvent-free microwave extraction
SPME	=	soild phase microextraction
Т	=	temperature
TCM	=	traditional Chinese medicine
TOF	=	time of fight
TPR	=	templated resin
v/v	=	volume by volume
w/w	=	weight by weight