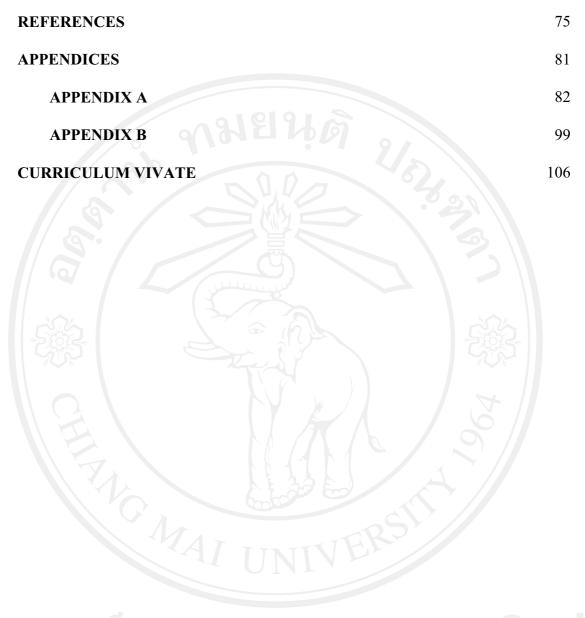
TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT (ENGLISH)	iv
ABSTRACT (THAI)	vi
LIST OF TABLES	xiii
LIST OF FIGURES	xvii
ABBREVIATIONS AND SYMBOLS	xx
CHAPTER I INTRODUCTION	
1.1 Molecularly Imprinted Polymers (MIPS)	1
1.1.1 Historical concept of MIPs	1
1.1.2 Molecular imprinting procedure	2
1.1.2.1 Covalent imprinting approach	4
1.1.2.2 Non-covalent imprinting approach	5
1.1.2.3 Semi-covalent imprinting approach	6
1.1.3 Constituents of MIP synthesis	6
1.1.3.1 Template	7
1.1.3.2 Functional monomers	8
1.1.3.3 Cross-linkers	10
1.1.3.4 Solvents (Porogens)	12
1.1.3.5 Initiators	12

1.2 Solid phase extraction	13
1.2.1 Solid phase extraction procedure	19
1.2.1.1 Conditioning	20
1.2.1.2 Sample loading	20
1.2.1.3 Washing	20
1.2.1.4 Drying	21
1.2.1.5 Elution	21
1.3 Molecularly imprinted polymers in solid phase extractions	22
1.4 Isoflavones	23
1.5 Analytical separation and detection methods for isoflavone	26
1.6 Aim of this research	27
CHAPTER II SYNTHESIS OF MOLECULARLY IMPRINTED	
POLYMER SELECTIVE TO GENISTEIN ISOFLAVONE USING	
FRAGMENT IMPRINTING TECHNIQUE	
2.1 Introduction	28
2.2 Experimental section	31
2.2.1 Chemicals and reagents	31
2.2.2 Instruments	31
2.2.3 Synthesis of imprinted and non-imprinted polymers	32
2.2.4 Binding studies of the polymers with their corresponding templates	32
2.2.5 Binding studies of the polymers with genistein	33
2.2.6 Characterization of the polymers	34

2.3 Results and Discussion	34
2.3.1 Synthesis of imprinted and non-imprinted polymers	34
2.3.2 Binding of the polymers with their corresponding	35
template	
2.3.3 Binding of the polymers with genistein	38
2.3.4 Characterization of the synthesized polymers	42
CHAPTER III OPTIMIZATION OF MOLECULARLY	
IMPRINTED SOLID PHASE EXTRACTION FOR GENISTEIN	
3.1 Introduction	45
3.2. Experimental section	47
3.2.1 Chemicals and Reagents	46
3.2.2 Instruments	47
3.2.3 Optimization of MISPE conditions	48
3.2.3.1 Optimization of loading conditions	48
3.2.3.2 Optimization of washing conditions	49
3.2.3.3 Optimization of elution conditions	50
3.2.4 Chromatographic analysis	51
3.2.5 MISPE selectivity	51
3.2.6 Application of the PIII-VP polymer to the extraction of	53
genistein from soybean extracts 3.2.6.1 Preparation of soybean extracts	0 53
3.2.6.2 MISPE of soybean extracts	53
3.2.6.3 LC-ESI-MS	54

3.3 Results and discussion	54
3.3.1 Optimization of MISPE conditions	54
3.3.1.1 Optimization of loading conditions	54
3.3.1.2 Optimization of washing conditions	56
3.3.1.3 Optimization of elution conditions	57
3.3.2 MISPE Selectivity	58
3.3.3 Extraction of genistein from soybean extract	60
CHAPTER IV STUDY OF MIXED-MODE MISPE FOR	
SELECTIVE EXTRACTION OF GENISTEIN	
4.1 Introduction	62
4.2 Experimental section	64
4.2.1 Chemicals and reagents	64
4.2.2 Instruments	64
4.2.3 Mixed-mode MISPE of PI-II-III and PIII-II-I	65
4.2.4 Selectivity of PI-II-III and PIII-II-I	67
4.2.5 Extraction of genistein from soybean extracts using	67
PI-II-III	
4.3 Results and discussion	68
4.3.1 MISPE of PI-II-III and PIII-II-I	68
4.3.2 Selectivity of PI-II-III and PIII-II-I	69
4.3.3 Extraction of genistein from soybean extracts using	70
PI-II-III	70
CHAPTER V CONCLUSION	72



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

xii

LIST OF TABLES

Table Page		
1.1	Approaches for imprinting molecules	4
1.2	Commercial sorbents in various types of extraction methods in SPE	16
2.1	Composition of the imprinted polymers	35
3.1	Loading conditions for optimization of MISPE procedure	49
3.2	Washing conditions for optimization of MISPE procedure	50
3.3	Elution conditions for optimization of MISPE procedure	50
3.4	Percentage recoveries, imprinting factor and selectivity factors from	59
	MISPE process of PI-VP, PII-AA and PIII-VP	
4.1	MIP cartridge order for mixed-mode MISPE	65
4.2	Percentage of genistein recoveries from mixed-mode MISPE process	68
	of PI-VP, PII-AA, PIII-VP, PI-II-III, PIII-II-I and their NIPs	
4.3	Percentage recoveries, imprinting factor and selectivity factors from	69
	mixed-mode MISPE process of PI-II-III and PIII-II-I	
A.1	Calibration curve of chromone (I) in ACN for binding studies	82
A.2	Calibration curve of 4-hydroxyphenylacetic acid (III) in ACN for	83
	binding studies	
A.3	Calibration curve of genistein in ACN for binding studies	84
A.4	The binding study of 0.30 mM chromone in ACN with 50 mg of	85
	PI-AA and its NIP	

A.5	The binding study of 0.30 mM chromone in ACN with 50 mg of	85
	PI-MAA and its NIP	
A.6	The binding study of 0.30 mM chromone in ACN with 50 mg of	85
	PI-VP and its NIP	
A.7	The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN	86
	with 50 mg of PIII-AA and its NIP	
A.8	The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN	86
	with 50 mg of PIII-MAA and its NIP	
A.9	The binding study of 0.30 mM 4-hydroxyphenylacetic acid in ACN	86
	with 50 mg of PIII-VP and its NIP	
A.10	The binding study of 0.10 mM genistein in ACN with 50 mg of	87
	PI-AA and its NIP	
A.11	The binding study of 0.10 mM genistein in ACN with 50 mg of	87
	PI-MAA and its NIP	
A.12	The binding study of 0.10 mM genistein in ACN with 50 mg of	87
	PI-VP and its NIP	
A.13	The binding study of 0.10 mM genistein in DCM with 50 mg of	88
	PI-AA and its NIP	
A.14	The binding study of 0.10 mM genistein in DCM with 50 mg of	88
	PI-MAA and its NIP	
A.15	The binding study of 0.10 mM genistein in DCM with 50 mg of	88
	PI-VP and its NIP	

A.16 The binding study of 0.10 mM genistein in ACN with 50 mg of	89
PII-AA and its NIP	
A.17 The binding study of 0.10 mM genistein in ACN with 50 mg of	89
PII-MAA and its NIP	
A.18 The binding study of 0.10 mM genistein in ACN with 50 mg of	89
PII-VP and its NIP	
A.19 The binding study of 0.10 mM genistein in DCM with 50 mg of	90
PII-AA and its NIP	
A.20 The binding study of 0.10 mM genistein in DCM with 50 mg of	90
PII-MAA and its NIP	
A.21 The binding study of 0.10 mM genistein in DCM with 50 mg of	90
PII-VP and its NIP	
A.22 The binding study of 0.10 mM genistein in ACN with 50 mg of	91
PIII-AA and its NIP	
A.23 The binding study of 0.10 mM genistein in ACN with 50 mg of	91
PIII-MAA and its NIP	
A.24 The binding study of 0.10 mM genistein in ACN with 50 mg of	91
PIII-VP and its NIP	
A.25 The binding study of 0.10 mM genistein in DCM with 50 mg of	92
PIII-AA and its NIP	
A.26 The binding study of 0.10 mM genistein in DCM with 50 mg of	92
PIII-MAA and its NIP	

A.27 The binding study of 0.10 mM genistein in DCM with 50 mg of	92
PIII-VP and its NIP	
A.28 Calibration curve of genistein in ACN using 2-napthol internal standard for MISPE from genistein standard	93
A.29 Calibration curve of quercetin in ACN using 2-napthol internal	94
standard for MISPE from quercetin standard	
A.30 Percentage of genistein recovery from PIII-VP and its NIP after	95
MISPE process in various using washing conditions	
A.31 Percentage of genistein recovery from PIII-VP after MISPE process	96
in various eluting conditions	
A.32 Calibration curve of genistein in ACN using 2-napthol as internal	97
standard for MISPE of genistein from soybean extracts	
A.33 The percentage of genistein recovery from MISPE process of	98
PIII-VP in soybean extracts	
A.34 The percentage of genistein recovery from MISPE process of	99
PI-II-III in soybean extracts	
B.1 Calculation of genistein relative content in soybean extract	103

LIST OF FIGURES

igu	re 1818180	Page
1.1	Schematic representation of the molecular imprinting procedure	3
1.2	Chemical structures of commonly used functional monomers in	9
	MIPs	
1.3	Chemical structures of commonly used cross-linkers in MIPs	11
1.4	Chemical structures of commonly used initiators in MIPs	13
1.5	The hardware and accessories for processing samples in SPE	19
1.6	SPE general procedures	21
1.7	The chemical structures of isoflavones commonly found in soybeans	25
2.1	Chemical structures of genistein, chromone (I), phloroglucinol (II)	30
	and 4-hydroxyphenylacetic acid (III)	
2.2	Percentage bound obtained from MIPs from chromone template and	37
	their corresponding NIPs	
2.3	Percentage bound obtained from MIPs from 4-hydroxyphenylacetic	37
	acid template and their corresponding NIPs	
2.4	Imprinting factor and % bound to genistein in ACN of the	39
	synthesized polymer obtained by using their corresponding template	
2.5	Imprinting factor and % bound to genistein in DCM of the	41
	synthesized polymer obtained by using their corresponding template	
2.6	SEM images of MIP and NIP of the selected polymers	43

2.7 FT-IR spectra of MIPs and NIPs of the selected polymers	44
3.1 General procedure of SPE process	46
3.2 The chemical structures of genistein and quercetin	52
3.3 The percentage of genistein recoveries from loading step with	55
difference washing solvents	
3.4 The percentage of genistein recoveries from washing step with	56
difference eluting solvents	
3.5 The percentage of genistein recoveries from eluting step with	57
different eluting solvents	
3.6 The HPLC chromatograms of soybean sample before and after	61
MISPE process	
4.1 The instrumental set up of MIP cartridges for mixed-mode MISPE	66
4.2 The HPLC chromatograms of soybean sample before and after	71
mixed-mode MISPE process	
A.1 Calibration curve of chromone (I) in ACN for binding studies	82
A.2 Calibration curve of 4-hydroxyphenylacetic (III) acid in ACN for	83
binding studies	
A.3 Calibration curve of genistein in ACN for binding studies	84
A.4 Calibration curve of genistein in ACN using 2-napthol internal	93
standard for MISPE from genistein standard	
A.5 Calibration curve of quercetin in ACN using 2-napthol internal	94

xviii

standard for MISPE from quercetin standard

A.6 Calibration curve of genistein in ACN using 2-napthol internal	97
standard for MISPE of genistein from soybean extracts	
B.1 Representative HPLC chromatogram of genistein in optimization of	99
MISPE process	
B.2 Representative HPLC chromatogram of genistein and quercetin in	99
the study of MISPE selectivity	
B.3 Structural formula and molecular weight of the main isoflavones	100
detected in soybean	
B.4 The HPLC-UV and MS chromatograms of soybean extract.	100
B.5 Mass spectrum of diadzein obtained from soybean extract	102
B.6 Mass spectrum of genistein obtained from soybean extract	102
B.7 Mass spectra of malonyl daidzein obtained from soybean extract	103
B.8 Representative HPLC chromatogram of soybean extracts; before	104
MISPE process; after loaded solution; after MISPE process using	
PIII-VP	
B.9 Representative HPLC chromatogram of soybean extracts; before	105 105
MISPE process; after loaded solution; after MISPE process using	
PI-II-III	

ABBREVIATIONS AND SYMBOLS

AA	Acrylamide
AIBN	Azobisisobutyronitrile
ASE	Accelerated solvent extraction
ACN	Acetonitrile
АсОН	Acetic acid
Avg	Average
BZP	Benzoyl peroxide
DAD	Diode-array detector
DCM	Dichloromethane
EGDMA	Ethyleneglycol dimethacrylate
EIC	Extracted ion chromatogram
ESI	Electrospray ionization
FA	Formic acid
FIT	Fragment imprinting technique
FT-IR	Fourier-transform infra-red spectroscopy
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
hrs	Hours hts reserved
LC	Liquid chromatography
MAA	Methacrylic acid
МеОН	Methanol

mg	Milligram
min	Minute
ml/min	Milliliter per minute
mM	Millimolar
mmol	Millimole
MS	Mass spectrometry
MW	Molecular weight
m/z	Mass to charge ratio
NIP	Non-imprinted polymer
ppm	Part per million
psi	Pond per square inch
SEM	Scanning electron microscopy
SD	Standard deviation
TIC	Total ion chromatogram
TEA	Triethylamine
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
°C	Degree celsius
λ	Wavelength Chiang Mai University
λ _{max}	The wavelength of maximum absorbance
α	Imprinting factor (calculated from % bound)
α'	Imprinting factor (calculated from % recovery)
3	Selectivity factor