

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (ENGLISH)	iv
ABSTRACT (THAI)	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
ABBREVIATIONS AND SYMBOLS	xiv
CHAPTER I INTRODUCTION	1
1.1 Development of High Performance Thin Layer Chromatographic Method for the Determination of Ketoconazole	3
1.1.1 Principles of High Performance Thin Layer Chromatography	3
1.2 Development of Ion Pair Liquid Chromatographic Method for the Determination of Ketoconazole	12
1.2.1 Principles of Ion Pair Liquid Chromatography	12
1.2.2 Literature Reviews	18
1.3 The Aim of Research	22
CHAPTER II EXPERIMENTAL	23
2.1 Development of High Performance Thin Layer Chromatographic Method for the Determination of Ketoconazole	23
2.1.1 Reagents and Chemicals	23
2.1.2 Apparatus and Instruments	24
2.1.3 Procedure	24
2.1.4 Method Validation	27

2.1.5 Application	29
2.2 Development of an Ion Pair Liquid Chromatographic Method for the Determination of Ketoconazole	30
2.2.1 Reagents and Chemicals	30
2.2.2 Apparatus and Instruments	31
2.2.3 Procedure	31
2.2.4 Method Validation	34
2.2.5 Application	35
CHAPTER III RESULTS AND DISCUSSION	36
3.1 Development of High Performance Thin Layer Chromatographic Method for the Determination of Ketoconazole	36
3.1.1 Optimization of the Experimental Conditions	36
3.1.2 Summary of the Optimum HPTLC Condition	37
3.1.3 Method Validation	38
3.1.4 Application	43
3.2 Development of IPLC Method for the Determination of Ketoconazole	46
3.2.1 Optimization of the Experimental Parameters	46
3.2.2 Summary of the Optimum IPLC Conditions	53
3.2.3 Validation of the Method	54
3.2.4 Application	57
CHAPTER IV CONCLUSION	59
4.1 High Performance Thin Layer Chromatographic Method	59
4.1.1 Optimum Conditions for Determining of Ketoconazole	59
4.1.2 Validation of the Method	60
4.1.3 Application	61

4.2 Ion Pair Liquid Chromatographic Method	62
4.2.1 Optimum Conditions for Determining of Ketoconazole	62
4.3.2 Validation of the Method	62
4.3.3 Application	63
REFERENCES	64
CURRICULUM VITAE	68

LIST OF TABLES

Table		Page
1.1	PMD Program Parameters	11
3.1	The R _f Values of Ketoconazole on Silica Gel 60 F ₂₅₄ Plate in Various Mobile Phases	37
3.2	Robustness of the Method	40
3.3	Precision Study for Ketoconazole	41
3.4	Recovery Studies on Ketoconazole	42
3.5	Comparison of HPTLC and Spectrophotometric Methods for the Determination of Ketoconazole in Shampoo and Pharmaceutical Formulations	44
3.6	Comparison of IPLC Analytical Column	52
3.7	Optimized Isocratic IPLC Condition for Analysis of Ketoconazole	53
3.8	Precision Study for Ketoconazole	56
3.9	Recovery Studies on Ketoconazole (Nizoral Sample)	57
3.10	Comparison of the proposed IPLC and the HPTLC Methods for the Determination of Ketoconazole in Shampoo and Pharmaceutical Formulations	58

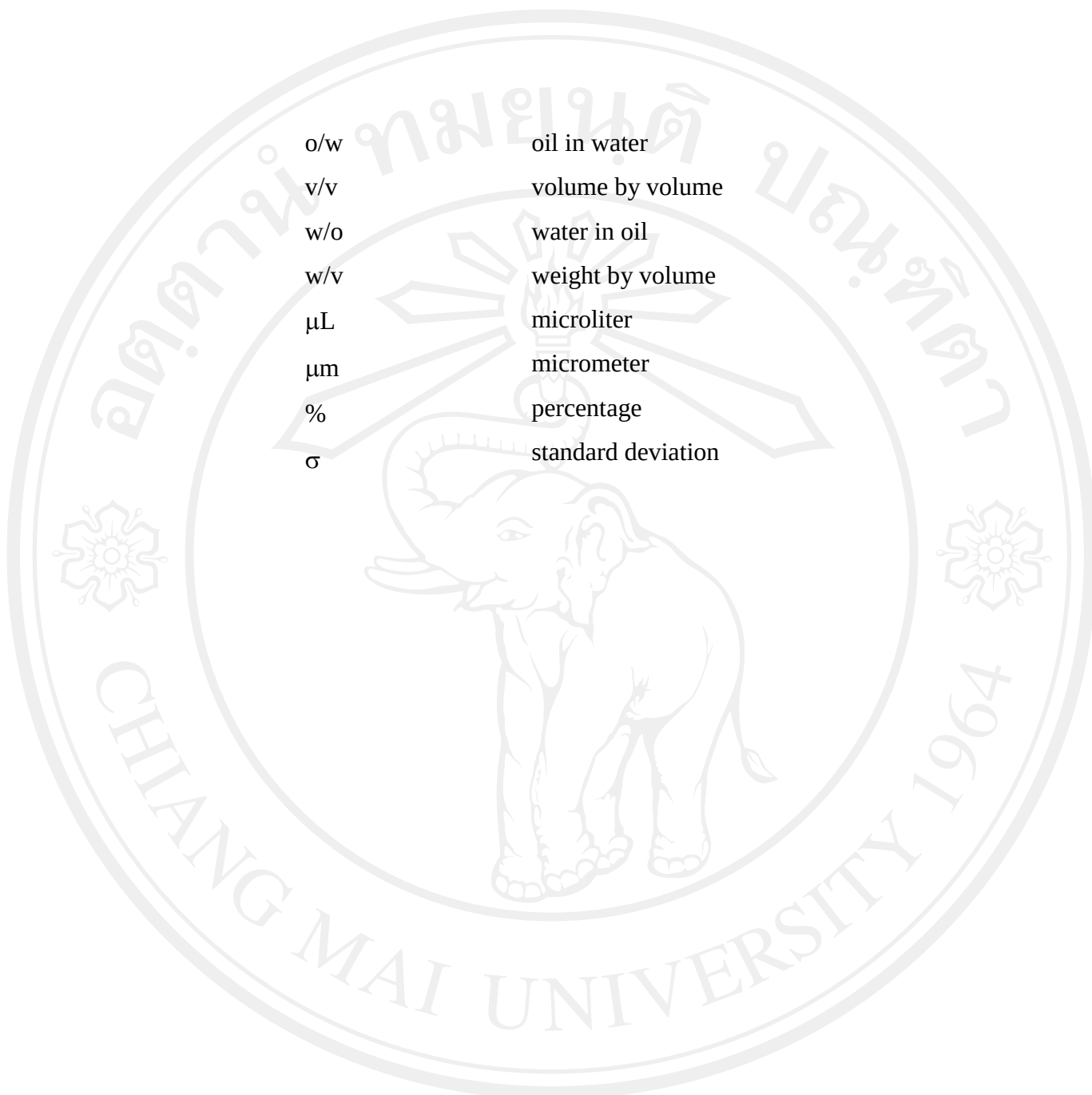
LIST OF FIGURES

Figure		Page
1.1	Structure of ketoconazole	1
1.2	CAMAG U-Kammer apparatus for radial high performance thin layer chromatography. (CAMAG Inc., New Berlin, Wis.)	4
1.3	Cross-sectional diagram of CAMAG UKammer. The HPTLC plate (1), measuring 50 × 50 mm, rests with its layer facing downward on the U-chamber body. (2) Elution solvent is fed to the center of the plate via a platinum-iridium capillary (3) of 0.2 mm internal diameter. Vapor phase, made up externally, may be passed through the chamber, in through the circular channel (4) and out through the center bore (5) before, during, and after chromatographic development. The direction of gas flow may also be reversed.	4
1.4	Schematic diagram of PMD developer. The thin layer plate is approximately 5 in. distant from and centered with respect to the radiator.	5
1.5	Programmer and developer for programmed multiple development (PMD).	5
1.6	Centered PMD. Selective evaporation along the chromatogram (vertical) center line, accomplished by heat or a nitrogen stream, induces horizontal solvent flow toward the center of the bed. This flow concentrates spots and counters horizontal spreading.	9
1.7	Influence of initial spot width (W_0) on spot behavior in programmed multiple development (PMD); resolution (R_s) between two spots of $R = 0.20$ and 0.21 as a function of the number of developments (n). The development program follows the pattern of IMD. Heavy emphasis on PMD solvent removal (dotted line) and light emphasis on PMD solvent removal (solid lines) are assumed.	10

1.8	Cation ion pairing interactions	14
1.9	Effects of ion pair reagent hydrophobicity and solvent concentration	14
3.1	Calibration graph for ketoconazole obtained by the HPTLC method (concentration range 3-20 mg L ⁻¹ of ketoconazole)	39
3.2	Densitogram of ketoconazole on silica gel 60 F ₂₅₄ in the solvent system, ethanol-acetone-1.0 mol L ⁻¹ H ₂ SO ₄ (80:10:10) with the R _f of 0.70.	43
3.3	Densitograms of ketoconazole samples on silica gel 60 F ₂₅₄ in the solvent system, ethanol-acetone-1.0 mol L ⁻¹ H ₂ SO ₄ (80:10:10).	45
3.4	The effect of SDS concentrations in the range 1-10 mM	47
3.5	The pH effect of mobile phase in the range 4.5-7.5	48
3.6	The effect of mobile phase composition	49
3.7	The effect of mobile phase flow rate; (a) = 0.5 mL min ⁻¹ , (b) = 0.8 mL min ⁻¹ , (c) = 1.0 mL min ⁻¹ and (d) = 2.0 mL min ⁻¹	51
3.8	The comparison of the analytical columns using three analytical columns.	52
3.9	Chromatogram of ketoconazole standard (a) and Linear calibration curve of ketoconazole in the concentration range of 5-500 mg L ⁻¹ (b)	55

ABBREVIATIONS AND SYMBOLS

AU	absorbance unit
cm	centimeter
°C	degree celsius
DI	deionize water
g	gram
h	hour
IPLC	ion pair liquid chromatography
i.d.	internal diameter
k	capacity or retention factor
kg	kilogram
L	liter
LOD	limit of detection
LOQ	limit of quantitation
M	molar (mol L ⁻¹)
mg	milligram
min	minute
mL	milliliter
mm	millimeter
nL	nanoliter
nm	nanometer
No.	number
PMD	programmed multiple development
R.S.D	relative standard deviation
S.D	standard deviation
SDS	sodium dodecyl sulfate
HPTLC	high performance thin layer chromatography
UV	ultraviolet

The background of the page features a large, faint watermark of the Chiang Mai University seal. The seal is circular, with an elephant in the center, a sunburst above it, and Thai script around the top. The English text "CHIANG MAI UNIVERSITY 1964" is written along the bottom arc of the seal.

o/w	oil in water
v/v	volume by volume
w/o	water in oil
w/v	weight by volume
μL	microliter
μm	micrometer
%	percentage
σ	standard deviation