

Penetration enhancers-containing vesicles for "Terbinafine HCl" skin delivery

A thesis submitted by

Sara Mahmoud Abdelsamie Mohamed

*Bachelor of Pharmaceutical Sciences, 2008, Ain Shams University Teaching
assistant, Department of Pharmaceutics and Industrial Pharmacy, Ain Shams
University*

*In the partial fulfillment of the requirements for the Master Degree in
Pharmaceutical Sciences (Drug technology)*

Under the supervision of

Prof. Dr. Omaila Ahmed Sammour

*Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy,
Ain Shams University*

Dr. Amany Osama Kamel

*Associate professor of Pharmaceutics and Industrial Pharmacy, Faculty of
Pharmacy, Ain Shams University*

**Ain Shams University
Faculty of Pharmacy
Department of Pharmaceutics and Industrial Pharmacy
2016**

List of Contents

Item	Page
List of abbreviations	I
List of tables	III
List of Figures	VI
Abstract	X
General introduction	1
Scope of work	18
Chapter I: Development of HPLC method for TBN HCl determination	
Introduction	19
Experimental	21
Methodology	23
I-Method development	23
a- Chromatographic system	23
b- Stock solution	23
c- Mobile phase composition	23
d- Factors influencing retention time	24
1- Influence of column temperature	24
2- Mobile phase composition	24
II- Method Validation	25
a- Linearity and range	26
b- Determination of limit of quantification (QL) and limit of detection (DL)	26
c- Specificity	27
d- Precision	27
1- Repeatability (intraday precision)	27

Item	Page
2- Reproducibility (Inter-day precision)	27
e- Accuracy	28
III- System suitability	28
Results and discussion	30
I- Method development	30
1- Influence of column temperature	30
2- Influence of mobile phase composition	33
II- Method Validation	36
a- Linearity and range	36
b- Determination of limit of quantification (QL) and limit of detection (DL)	38
c- Specificity	38
d- Precision	38
e- Accuracy	39
III - System suitability	41
Conclusions	43
Chapter II: Preparation and characterization of TBN HCl loaded vesicles.	
Introduction	44
Experimental	52
Methodology	54
I- Determination of λ_{\max} of TBN HCl	54
II- Construction of the calibration curves of TBN HCl in different hydro-alcoholic solutions	54

Item	Page
III- Preparation of Ethosomes	54
A) Optimization studies for the preparation of ethosomes	55
1. Effect of homogenization time	55
2. Effect of Phospholipid concentration	55
3. Effect of the hydro-alcoholic solution concentration	55
B) Factorial design of the experiment	56
IV- Preparation of TBN HCl loaded PEVs	58
V- Characterization of vesicles	59
1- Particle size and PDI	59
2- Measurement of zeta potential	60
3- Determination of entrapment efficiency percent	60
4- Vesicles visualization by transmission electron microscopy (TEM)	60
5- Differential scanning calorimetry (DSC)	61
6- Deformability index measurement	61
VI- <i>Ex vivo</i> studies	62
i. Determination of Terbinafine HCl solubility in release medium	62
ii. <i>Ex vivo</i> skin permeation and deposition studies	62
iii. Tape stripping studies	64
iv. Skin image by fluorescence microscopy	64
v. Skin image by confocal laser scanning microscopy (CLSM)	65
VII- Stability study	65
VIII- Statistical analysis	66

Item	Page
Results and discussion	67
I- Determination of λ_{\max} of TBN HCl	67
II- Construction of the calibration curves of TBN HCl in different hydro-alcoholic solutions	69
III- Preparation of Ethosomes	74
A) Preliminary studies for the preparation of ethosomes	75
1. Effect of homogenization time	75
2. Effect of Phospholipid concentration	76
3. Effect of the hydro alcoholic solution concentration	78
B) Factorial design of the experiment	80
1- Particle size and PDI analysis	80
2- Zeta potential	84
3- Entrapment efficiency percent	86
IV- Preparation of TBN HCl loaded PEVs	88
A) Effect of ethanol on the characteristics of TBN HCl loaded PEVs	88
B) Factorial design of PEVs prepared with ethanol	90
i. Measurement of PS and PDI	91
ii. Measurement of ZP	94
iii. Determination of entrapment efficiency percent	96
V- Transmission electron microscopy (TEM)	98
VI- Differential scanning calorimetry	100
VII- Deformability index determination	101
VIII- <i>Ex vivo</i> studies	103

Item	Page
1- Determination of Terbinafine HCl solubility in release medium	103
2- <i>Ex vivo</i> skin permeation and deposition studies	104
3- Tape stripping studies	112
4- Skin image by fluorescence microscopy	117
5- Skin image by confocal laser scanning microscopy (CLSM)	119
IX- Stability studies	121
Conclusions	125
Chapter III:	
Preparation and characterization of TBN HCl vesicular gels	
Introduction	127
Experimental	131
Methodology	133
I-Preparation and evaluation of TBN HCl ethosomal gels	133
a- Preparation of TBN HCl ethosomal carbopol 934 gel	133
b- Preparation of TBN HCl ethosomal HEC gel	133
c- Preparation of TBN HCl ethosomal chitosan gel	133
d- Preparation of TBN HCl ethosomal pluronic F127 gel	134
II-Rheological studies and viscosity determination	134
III- <i>Ex vivo</i> permeation and deposition studies for TBN HCl ethosomal gels	135

Item	Page
IV- Preparation and evaluation of TBN HCl PEVs gels	135
V- Characterization of vesicular gels	135
a- Particle size measurement	135
b- Determination of drug content	135
VI- Statistical analysis	136
Results and discussion	137
I- Rheological studies and determination of gels viscosity	137
II- <i>Ex vivo</i> permeation and deposition studies for TBN HCl vesicular gels	139
III- Characterization of vesicular gels	142
a- Particle size measurement	142
b- Determination of drug content	143
Conclusions	144
Chapter IV: <i>In vivo</i> evaluation of TBN HCl loaded vesicles and vesicular gels	
Introduction	145
Experimental	149
Methodology	151
I- Microbiological evaluation	151
II- <i>In vivo</i> skin irritation evaluation	151
III- <i>In vivo</i> animal evaluation	152
• Test organism and preparation of fungal inoculums	153
• Induction of cutaneous candidal infection	153

Item	Page
• Construction of calibration curve for McFarland scale	154
IV- <i>In vivo</i> clinical evaluation	155
V- Statistical analysis	157
Results and discussion	158
I- Microbiological evaluation	158
II- <i>In vivo</i> skin irritation evaluation	161
III- <i>In vivo</i> animal evaluation	162
IV- <i>In vivo</i> clinical evaluation	166
Conclusions	168
Summary	169
References	177
Ethical committee	217
Arabic Summary	1

List of Abbreviations

Symbol	Abbreviation
ANOVA	Analysis of variance
AT	Ambient temperature
<i>C. albicans</i>	<i>Candida albicans</i>
c.f.	Compare for
CFU	Colony forming unit
Cin	Cineole
CLSM	Confocal laser scanning microscope
Conc.	Concentration
DI	Deformability index
DL	Detection limit
DSC	Differential scanning calorimetry
EE%	Entrapment Efficiency percent
ER	Enhancement ratio
FDA	Food and drug administration
FM	Florescent microscope
GRAS	Generally regarded as safe
HEC	Hydroxy ethyl cellulose
HETP	Height equivalent to theoretical plates
HPC	Hydroxy propyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
HT	Homogenization time
ICH	International conference of harmonization
Lab	Labrasol
LAC	Local accumulation capacity
Limo	Limonene
LUVs	Large uni-lamellar vesicles
MIC	Minimum inhibitory concentration
MLVs	Multi-lamellar vesicles
NCs	Nanocarriers
OECD	Organization for Economic Co-operation and Development,
PDI	Poly dispersity index
PE/s	Penetration enhancer/s
PEVs	Penetration enhancer containing vesicles
PEG	Polyethylene glycol
PG	Propylene glycol

Symbol	Abbreviation
PL/s	phospholipid/s
PS	Particle size
QL	Quantification limit
RLD	Reference listed drug
RSD	Relative standard deviation
SAA	Surfactant
SC	Stratum corneum
SCR	Size change rate
SD	Standard deviation
SLNs	Solid lipid nanoparticles
SUVs	Small uni-lamellar vesicles
TBN HCl	Terbinafine hydrochloride
TEM	Transmission electron microscope
T _m	Transition temperature
Trans	Transcutol
UV	Ultraviolet
ZP	Zeta potential

List of Tables

Table number	Table name	Page
1	Different mobile phase compositions for method optimization	25
2	Influence of column temperature on the retention time of TBN HCl	31
3	Influence of mobile phase composition on the retention time of TBN HCl	35
4	Results for TBN HCl calibration curve by HPLC method	37
5	Results for intraday precision	39
6	Results for inter-day precision	39
7	Accuracy results	40
8	Summary of validation data obtained for the developed HPLC method for TBN HCl assay	42
9	Composition of ethosomal formulations to study the effect of changing various formulation parameters	56
10	Factors and levels used for factorial design for the preparation of TBN HCl loaded ethosomes	57
11	Composition of ethosomes according to factorial design	57
12	Factors and levels used for the preparation of TBN HCl loaded PEVs	59
13	Composition of different PEVs for factorial design	59
14	Relationship between concentration of TBN HCl and absorbance at λ_{\max} 283 nm in 30% ethanol solution	70

Table number	Table name	Page
15	Relationship between concentration of TBN HCl and absorbance at λ_{\max} 283 nm in 40% ethanol solution	71
16	Relationship between concentration of TBN HCl and absorbance at λ_{\max} 282.5 nm in 50% ethanol solution	73
17	Effect of various formulation parameters on PS, ZP and EE% of TBN HCl loaded ethosomes	77
18	Results for PS, PDI, ZP and EE% for formulae (F1-F8) in factorial design experiment	82
19	ANOVA for PS response of TBN HCl loaded ethosomes according to the factorial design	82
20	ANOVA for PDI response of TBN HCl loaded ethosomes according to the factorial design	83
21	ANOVA for ZP response of TBN HCl loaded ethosomes according to the factorial design	85
22	ANOVA for EE% response of TBN HCl loaded ethosomes according to the factorial design	87
23	Results of PS , PDI, ZP and EE% for TBN HCl loaded PEVs \pm SD, n=3	90
24	ANOVA for PS response of TBN HCl loaded PEVs according to the factorial design	92
25	ANOVA for PDI response of TBN HCl loaded PEVs according to the factorial design	92
26	ANOVA for ZP response of TBN HCl loaded PEVs according to the factorial design	95
27	ANOVA for EE% response of TBN HCl loaded PEVs according to the factorial design	97

Table number	Table name	Page
28	Results for deformability index determination for the prepared vesicles	103
29	<i>Ex vivo</i> permeation and deposition data of different formulae, n=6 \pm SD	108
30	<i>Ex vivo</i> tape stripping data, \pm SD, n=6	113
31	Results for PS and calculation of SCR through three months stability studies	124
32	Results for <i>ex vivo</i> studies for vesicular gels	142
33	PS and drug content of vesicular chitosan gels	143
34	Construction of McFarland scale	155
35	Results for the zones of growth inhibition of <i>C. albicans</i> \pm SD, n=3	159
36	Results for calibration curve of McFarland scale for detection of fungal count	164
37	Results of success and failure cases for the clinical study	167

List of Figures

Figure number	Figure name	Page
I	Optical microscopy of a histological section of skin	1
II	Routes of drug penetration through skin	4
III	Schematic diagram of a) static franz diffusion cell, b) side by side diffusion cell and c) flow- through diffusion cell	11
IV	TBN HCl chemical structure	16
V	Proposed mechanism of action of ethosomes	46
VI	Structures of PG (A) and transcutol (B)	47
VII	Structures of different terpenes molecules	49
VIII	Formation of vesicular gel	127
IX	Cubic structure of pluronic F 127 gel	140
1	Chromatogram showing TBN HCl peak at 35 min. Column temperature was 25 °C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (50:25:25)(v/v).	31
2	Chromatogram showing TBN HCl sharp peak at 25 min. Column temperature was 35 °C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (50:25:25)(v/v)	32
3	Chromatogram showing TBN HCl peak at 1 min, interfering with peaks of impurities. Column temperature was 45 °C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (50:25:25)(v/v).	32
4	Chromatogram showing no obvious drug peak. Column temperature was 35 °C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (75:12.5:12.5)(v/v).	33

Figure number	Figure name	Page
5	Chromatogram showing TBN HCl sharp peak at 15 min. Column temperature was 35°C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (50:30:20)(v/v).	34
6	Chromatogram showing broad peak of TBN HCl at 4 min, interfering with peaks of impurities. Column temperature was 35°C and mobile phase composition was acetonitrile: methanol: 0.01M dipotassium hydrogen phosphate pH 7.4 (50:35:15)(v/v).	34
7	Calibration curve for the developed HPLC method.	37
8	Ultraviolet spectrum scan of TBN HCl in 30% ethanol	67
9	Ultraviolet spectrum scan of TBN HCl in 40% ethanol	68
10	Ultraviolet spectrum scan of TBN HCl in 50% ethanol	68
11	Calibration curve of TBN HCl in 30% ethanol solution in at λ_{\max} 283nm	70
12	Calibration curve of TBN HCl in 40% ethanol solution at λ_{\max} 283 nm	72
13	Calibration curve of TBN HCl in 50% ethanol solution at λ_{\max} 282.5 nm	74
14	Plots for the main effect of different factors on PS of TBN HCl loaded ethosomes: (A) Ethanol concentration and (B) Drug concentration	83
15	Interaction plot of different factors on PS response of TBN HCl loaded ethosomes	84
16	Plots for the main effect of different factors on ZP of TBN HCl loaded ethosomes: (A) Ethanol concentration and (B) Drug concentration	85
17	Interaction plot of different factors on ZP response of TBN HCl loaded ethosomes	86
18	Plots for the main effect of different factors on EE% of TBN HCl loaded ethosomes: (A) Ethanol concentration and (B) Drug concentration	87
19	Interaction plot of different factors on EE% response of TBN HCl loaded ethosomes	88