PHARMACOKINETICS AND PHARMACODYNAMICS MODELING OF CLOZAPINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH SCHIZOPHRENIA SPECTRUM DISORDERS USING PHARMACOMETRICS APPROACH

ALBITAR ORWA

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by

ALBITAR ORWA

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The future has been an unpredictable journey, a non-pharmacometrician once said. The core principle of the Bayes' Theorem is that our actions and decisions account for what we have become. When seeking the truth through the scientific method, research and exploration become a state of mind.

This is the very single page where all the contributions, assistance, and guidance in the work that follows can be declared. It has been a great proud to work with my mentor and supervisor, Dr. Siti Maisharah Sheikh Ghadzi. Besides the vast knowledge, the unlimited help she provided has opened priceless current and future opportunities. She always welcomed all the new and novel ideas introducing all the improvements and entrusting me to present in front of potential international collaborators. I sincerely hope our work together outlive the PhD.

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LIST OF SYMBOLS

1-β	Study power
5-HT2	5-hydroxytryptamine
A	The change rate of normal physiology
A_0	The baseline physiological parameter
ADR_{obs}	Observed adverse effect
ADR_{pred}	Predicted adverse effect
α	Significance level
AMP	Amplitude of the adverse effect
A(t)	Adverse effect time course
A(x)	Amounts in compartment x
AUC	Area under the concentration time curve
bpm	Beats per minute
BXPAR	Box-cox parameter
cov_i	Individual value of the covariate
cov_{median}	Median value of the continuous covariate
C	Carbon
°C	Celsius
C_0	Trough concentration
cAUC50	Cumulated AUC required to attain half of E_{max}
CL	Clearance
CL_i	Individualized clearance
C_{added}	Known concentration of the standard
C_{enz}	Concentration in the enzyme compartment
C_f	Concentration in the organic phase
C_i	Concentration in the aqueous phase

C_{found}	Concentration after adding a known amount of the standard to the actual samples
C_{max}	Maximum concentration
C_{min}	Minimum concentration
C_{obs}	Observed concentration
C_{pred}	Predicted concentration
C_{real}	Actual concentration of the sample
C_x	Random concentration or concentration at time x
CTAB	Cetyl trimethyl ammonium bromide
CV	Coefficient of variation
D	Dopamine
Δ	The difference between two groups that the sample size can detect
E	The time course of drug pharmacological adverse effects
EDTA	Ethylene diamine tetra acetic acid
E_{max}	Maximum effect
ε	Residual unexplained variability
ER	Extraction ratio
η_i	Inter-individual random effects
F	Bioavailability
FM	Fraction metabolized
g	Gravitational force
h	Hour
h(t)	Hazard at time t
HCl	Hydrochloric acid
HR	Hazard ratio
K_a	Absorption rate constant
K_{AE}	Rate constant of deterioration due to the adverse effect
K_e	Elimination rate constant

K_{Rec}	Rate constant of recovery from the adverse effect
K_{x0}	Elimination rate constants from compartment x
K_{xy}	Elimination rate constant from compartment x to compartment y
L	Liter
λ_0	Scale parameter of the Weibull hazard
λ_{PR}	Lambda of box-cox on baseline
L(t)	Survival
m	Calibration curve slope
M	Muscarine
min	Minute
µL	Microliter
µm	Micrometer
mg	Milligram
mL	Milliliter
mmol	Millimole
mol/L	Mole
n	Sample size
n_f	Amount in the organic phase
n_i	Amount in the aqueous phase
ng	Nanogram
nm	nanometer
NaCl	Sodium chloride (salt)
NaOH	Sodium hydroxide
OS	Offset
p	p-value
P(PR)	Subpopulation Tachycardia
P(WG&AD)	Subpopulation Weight gain & ANC drop

P(AE)	Subpopulation All AEs
%	Percent
P_E	Probability of an event
P_A	Proportion of group A in the sample size
P_B	Proportion of group B in the sample size
pH	Potential hydrogen
pK_a	Acid dissociation constant
PT	Peak time of the surge function
PW	Peak width of the surge function
Q	Intercompartmental clearance
QH	Total liver blood flow
ω^2	Variance of inter-individual random effects distribution
r^2	Regression coefficient
R	Correlation coefficient
®	Registered trademark
S	Progression rate of the natural history of the disease without any drug effect
SE	Standard error
σ^2	Variance of residual unexplained variability distribution
SL	Slope of the adverse effect
S(t)	The time course of disease status
S_0	The baseline disease status
SDS	Sodium dodecyl sulfate
SWT	Switching time to another slop in the piecewise linear function
t	Time
θ	Adverse effect or pharmacokinetic parameter population estimate
θ_i	Adverse effect or pharmacokinetic parameter individual estimate
θ_{cov}	Covariate coefficient of an individual

t_i	Observation time
t_{mean}	Mean observation times
T-lag	Absorption lag time
$T_{1/2}$	Half-life
τ	Shape parameter of the Weibull hazard
TFA	Trifluoroacetic acid
T_{max}	Maximum concentration time
v	volume
V_f	volume of organic phase
V_i	Volume of aqueous phase
V_c	Volume of distribution of the central compartment
V_x	Volume of distribution in compartment x
Vp	Volume of distribution of the peripheral compartment
Z_α	The Z number corresponding to the α significance level
Z_β	The Z number corresponding to the β study power

LIST OF ABBREVIATIONS

95% CI	95% confidence intervals
AAP	Atypical antipsychotics
ABC	ATP-binding cassette
AD	ANC drop
Add	Additive error
ADR	Adverse drug reaction
AGNP	Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie
aHR,	Adjusted hazard ratio
AIC	Akaike information criterion
AM	Amperometric
ANC	Absolute neutrophils count
BMI	Body mass index
BNSS	Brief Negative Symptom Scale
BPRS	Brief Psychiatric Rating Scale
BPRS-A	Brief Psychiatric Rating Scale-Anchored
BSA	Body surface area
CATIE	Clinical Antipsychotic Trials for Intervention Effectiveness
CLZ	Clozapine
CGI	Clinical global impression
CGI-S	CGI severity
CGI-I	CGI improvement
CMC	Critical micelle concentration
Cov	Covariate
CTDD	Clozapine cumulative dose achieved after six months
CUtLASS	UK Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study
CYP450	Cytochrome P450
DBP	Diastolic blood pressure
df	Mean difference
DLLME	Dispersive liquid-liquid microextraction
DoTS	Dose relatedness, timing, and patient susceptibility
Dox	Doxepin

ECG	Electrocardiography
EPS	Extrapyramidal symptoms
FDA	Food and Drug Administration
FIN11	11 years follow-up in a study in Finland
FMO3	Flavin monooxygenase 3
FOCE-I	First-order conditional estimation method with interaction
GAF	Global Assessment of Functioning
GC	Gas chromatography
GOF	Goodness of fit
GERD	Gastro-esophageal reflux disease
HALO	Haloperidol
HPLC	High-performance liquid chromatography
IBF	Inverse Bateman function
IIV	Inter-individual variability
InterSePT	International Suicide Prevention randomized trial
iWRES	Individual weighted residuals
KM-VPC	Kaplan–Meier-Visual predictive check
LDR	Linear dynamic range
LLE	Liquid-liquid extraction
LLP	Log-likelihood profiling
LOD	Limit of detection
LLOQ	Lower limit of quantification
LPME	liquid-phase microextraction
MDR 1	Multidrug resistance protein 1
MPO	Myeloperoxidase
MS	Mass spectroscopy
MDR	Multidrug resistance protein
mix-VPC	Mixture visual predictive check
NA	Not available
NCLZ	Norclozapine
NMDA	N-methyl-d-aspartate
NONLIN	Nonlinear regression
NONMEM	Nonlinear mixed-effect modeling
NPC	Numerical predictive check

NPDE	Normalized prediction distribution error
NPML	Nonparametric maximum likelihood
NPRA	National Pharmaceutical Regulatory Agency
NWPRI	Normal–inverse Wishart distribution
OFV	Objective function value
PANSS	Positive and negative syndrome scale
Pant	Concomitant intake of pantoprazole
PBPK	Physiological based pharmacokinetic models
pcVPC	Prediction-corrected visual predictive check
PKPD	Population pharmacodynamic and pharmacokinetic model
PPI	Proton pump inhibitor
PR	Pulse rate
Pro	Proportional error
PUD	Peptic ulcer disease
R&I	Research & Innovation
RCTs	Randomized control trials
RSD	Relative standard deviation
RSE	Relative standard errors
SA-DLLME	Surfactant-assisted dispersive liquid-liquid microextraction
SANS	The Scale for the Assessment of Negative Symptoms
SAPS	The Scale for the Assessment of Positive Symptoms
SBP	Systolic blood pressure
SCID	Structural Clinical Interview for DSM-V
SD	Standard deviation
SSD	Schizophrenia spectrum disorders
SSRI	Selective serotonin reuptake inhibitors
SIR	Sampling-importance resampling method
SOHO	Schizophrenia Outpatient Health Outcome
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SSD	Schizophrenia spectrum and other psychotic disorders
TC	Tachycardia
TDM	Therapeutic drug monitoring
TRRIP	Treatment Response and Resistance in Psychosis

TRS	Treatment resistance schizophrenia
UGT	Uridine diphosphateglucuronosyl-transferase
UK	United Kingdom
USA	United States of America
USP	United States Pharmacopeia
UV	Ultraviolet
VPC	Visual predictive check
WG	Weight gain
Wt	Weight

LIST OF APPENDICES

PEMODELAN FARMAKOKINETIK DAN FARMAKODINAMIK BAGI CLOZAPINE DALAM SUKARELAWAN SIHAT DAN PESAKIT GANGGUAN SPEKTRUM SKIZOFRENIA MENGGUNAKAN PENDEKATAN FARMAKOMETRIK

ABSTRAK

Clozapine ialah drug barisan pertama untuk skizofrenia rintang-rawatan. Walau bagaimanapun, ia mempunyai metabolisme yang kompleks, hubungan pendedahan-tindak balas yang tidak jelas, dan potensi kesan advers (ADRs). Kajian semasa ini bertujuan untuk membangunkan kaedah analisis dan model berasaskan farmakometrik untuk farmakokinetik dan farmakodinamik bagi clozapine dan norclozapine dalam kalangan sukarelawan sihat dan pesakit gangguan spektrum skizofrenia (SSD). Pengekstrakan mikro cecair-cecair penyebaran berbantu-surfaktan ditambah dengan HPLC-UV telah digunakan untuk penentuan clozapine dan norclozapine selepas mengoptimumkan faktor-faktor eksperimen. 270 titik data yang diperoleh daripada 33 sukarelawan sihat selepas menerima satu dos 12.5 mg clozapine telah digunakan dalam pembangunan model farmakokinetik separa fisiologi yang menggabungkan metabolisme clozapine pra-sistemik. Untuk menilai interaksi clozapine dengan pantoprazole, sebuah reka bentuk kajian rawak, silang, dan label terbuka telah dilaksanakan. 12 sukarelawan telah diberikan clozapine sahaja; kemudian clozapine selepas lima hari pantoprazole 40 mg atau sebaliknya, dipisahkan dengan tempoh basuh habis. Sementara itu, data klinikal telah diekstrak daripada 116 rekod perubatan pesakit SSD di Hospital Besar Pulau Pinang, dengan purata susulan retrospektif selama 306 minggu. Beberapa model kemandiran parametrik telah dinilai untuk model masa-ke-pembaikan simptom positif setelah

clozapine dimulakan. Selain itu, ADRs diwakili sebagai fungsi masa dengan menggabungkan model campuran untuk menggambarkan kerentanan individu. Kaedah analisis menunjukkan prestasi yang boleh diterima dengan had pengkuantitian sebanyak 0.9 dan 0.4 ng/mL untuk clozapine dan norclozapine, masing-masing. Model clozapine dua-kompartmen dan norclozapine dua-kompartmen dengan penyerapan dan penyingkiran tertib pertama adalah paling sesuai dengan data. Bangsa Kulit Hitam Afrika mempunyai penyingkiran clozapine yang lebih rendah, Bangsa Kulit Putih mempunyai penyingkiran clozapine yang lebih tinggi, isipadu sebaran meningkat dengan berat badan yang lebih tinggi, dan pantoprazole menurunkan kepekatan clozapine, dan Cmax, Tmax, dan AUC (0-8) bagi clozapine dan norclozapine. Analisis masa-ke-peristiwa diterangkan dengan terbaik oleh fungsi bahaya Weibull. Bahaya bagi pembaikan simptom meningkat pada usia yang lebih muda, dengan rawatan serentak antipsikotik atipikal, dan dos kumulatif clozapine yang lebih tinggi. Takikardia yang disebabkan oleh clozapine, pertambahan berat badan, dan neutropenia diterangkan dengan baik oleh ofset, linear sesecebis dan dua fungsi pusuan, masing-masing. 8.5% pesakit mempunyai semua ADRs, 24.7% mengalami penambahan berat badan dan neutropenia, dan 45.6% mengalami takikardia. Kaedah analisis yang dibangunkan adalah boleh dilaksanakan dan cepat, yang mana ia memberi faedah kepada pembangunan pemonitoran drug terapeutik bagi clozapine pada masa akan datang. Selain itu, ini adalah kajian pertama untuk membangunkan model berasaskan farmakometrik yang menangani metabolisme kompleks clozapine, interaksinya dengan pantoprazole, peramal tindak balasnya, dan ADRs dalam pendekatan baharu dengan potensi aplikasi dalam pembangunan drug baharu.

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ABSTRACT

Clozapine is the first-line medication for treatment-resistant schizophrenia. However, it has complex metabolism, an unclear exposure-response relationship, and potential adverse drug reactions (ADRs). The present study aimed to develop an analytical method and pharmacometrics-based models for clozapine and norclozapine pharmacokinetics and pharmacodynamics in healthy volunteers and schizophrenia spectrum disorder (SSD) patients. A surfactant-assisted dispersive liquid-liquid microextraction coupled with HPLC-UV was used for clozapine and norclozapine determination after optimizing experimental factors. 270 data points obtained from 33 healthy volunteers after receiving a single dose of 12.5 mg clozapine were utilized in the semi-physiological pharmacokinetic model development incorporating the pre-systemic clozapine metabolism. To assess clozapine interaction with pantoprazole, a randomized, crossover, open-label study design was implemented. 12 volunteers were given clozapine alone; then clozapine after five days of 40 mg pantoprazole or vice versa, separated by a washout period. Meanwhile, clinical data were extracted from 116 SSD patients' medical records in Penang General Hospital, with a mean retrospective follow-up of 306 weeks. Several parametric survival models were evaluated for the time-to-positive symptoms improvement model after the initiation of clozapine. Moreover, the ADRs were represented as functions of time by incorporating a mixture model to describe

individual susceptibility. The analytical method showed acceptable performance with a limit of quantification of 0.9 and 0.4 ng/mL for clozapine and norclozapine, respectively. A two-compartment clozapine and two-compartments norclozapine with first-order absorption and elimination model best fit the data. African Black race had lower clozapine clearance, Caucasians had higher clozapine clearance, the volume of distribution increased with higher bodyweight, and pantoprazole decreased clozapine concentrations, clozapine and norclozapine C_{max}, T_{max}, and $AUC_{(0-8)}$. The time-to-event analysis was best described by a Weibull hazard function. The hazard of symptoms improvement increased at a younger age, with the concomitant treatment of atypical antipsychotics and higher clozapine cumulative doses. Clozapine-induced tachycardia, weight gain, and neutropenia were best described by an offset, a piecewise linear, and a two-surge function, respectively. 8.5% of the patients had all the ADRs, 24.7% had weight gain and neutropenia, and 45.6% experienced tachycardia. The analytical method was feasible and fast, which is an advantage of the future development of therapeutic drug monitoring of clozapine. Additionally, this is the first study to develop pharmacometrics-based models addressing clozapine complex metabolism, its interaction with pantoprazole, predictors of its response, and its ADRs in a new approach with potential applications in new drug development.

CHAPTER 1

INTRODUCTION

1.1 Schizophrenia spectrum and other psychotic disorders

Schizophrenia spectrum and other psychotic disorders (SSD) are chronic and severe diseases (Lehman et al., 2004) that include schizophrenia, schizotypal (personality) disorder, delusional disorder, brief psychotic disorder, schizophreniform disorder, schizoaffective disorder, and substance/medication-induced psychotic disorders, and psychotic disorders due to another medical condition (DSM-5, 2013). They are characterized by positive symptoms such as delusions, hallucinations, disorganized thinking, abnormal motor behavior, and negative symptoms such as asociality and anhedonia (DSM-5, 2013). Eugen Bleuler, a Swiss psychiatrist, was the first to use the term “schizophrenia”, which was derived from the Greek “schizo” (split) and “phren” (mind) to describe a loosening of association rather than a split personality (Bleuler, 1950).

By 2020, schizophrenia had affected around 20 million people worldwide, a major cause of disability (World Health Organisation, 2020) with a high unemployment rate of 80-90% and a reduced life expectancy of 10-20 years (M. J. Owen et al., 2016). The global burden of schizophrenia was recorded to be the highest among East, South, and Southeast Asian countries (He et al., 2020). In Malaysia, the prevalence of schizophrenia was recorded as 106,900 in 2019 (Ferrari, 2022). total estimated treated schizophrenia cases in 2015 were 15,104, with an economic burden of USD 100 million (Teoh et al., 2017). There is inadequate support for schizophrenia patients. More resources should be allocated to improve their condition and reduce the economic burden, including public health education, health care services, and other means to enhance schizophrenia patients’ conditions

and avoid the decreased productivity associated with this disease in the long run (Teoh et al., 2017). In a large meta-analysis, the incidence rates of schizophrenia were 10 in females and 15 in males per 100,000 populations per year, with a lifetime prevalence of 0.46% is prevalent across diverse populations and cultures (McGrath et al., 2008).

1.2 Schizophrenia symptoms

Schizophrenia symptoms are described below based on (DSM-5, 2013):

1.2.1 Positive symptoms

1.2.1(a) Delusions

Delusion is one of the major manifestations of schizophrenia positive symptoms (Bell et al., 2021). It is defined as a fixed belief that is not amenable to change in the light of conflicting evidence. The types of delusion are summarized as the following statements.

1. Persecutory delusion is the most common delusion, which is a belief that one is threatened to be harmed by other individuals, groups, or organizations.
2. Referential delusion is a belief that gestures, comments, or environmental cues are directed at oneself.
3. Grandiose delusion is one's belief that he or she has exceptional abilities, wealth, or fame.
4. Erotomanic delusion is an individual false belief that someone else is in love with him or her.
5. Nihilistic delusion is the conviction that a major catastrophe would occur.
6. Somatic delusions focus on preoccupations with organ function and health.

1.2.1(b) Hallucinations

Hallucination is an involuntary, clear, and vivid perception-like experience that occurs without an external stimulus. Auditory is the most common type of hallucinations. However, it can also appear in any sensory form as well as when falling asleep (hypnagogic hallucination) or waking up (hypnopompic hallucination).

1.2.1(c) Disorganized thinking (speech)

Disorganized thinking in terms of speech can be observed as in the statements below.

1. Derailment (loose association) is switching from one topic to another.
2. Tangentiality is responding to questions with irrelevant answers.
3. Incoherence (word salad) is severe and incomprehensible disorganization of speech that resembles receptive aphasia.

1.2.1(d) Grossly disorganized or abnormal motor behavior

These conditions can range from childhood "silliness" to unpredictable agitation and might include *catatonic behavior*, which is a significant reduction in environment reactivity. The statements below summarize the types of grossly disorganized or abnormal motor behavior.

1. Negativism is resistance to instructions.
2. Mutism and stupor are a complete absence of verbal and motor response.
3. Catatonic excitement includes a pointless increase in motor activity with no apparent purpose.

1.2.2 Negative symptoms

Negative symptoms can be observed as the following:

1. Diminished emotional expression, which is a decreased expression of facial emotions, the intonation of speech, head movements, and eye contact that gives the emotional emphasis to speak normally.
2. Avolition, which represents diminution in motivated self-initiated purposeful activities
3. Alogia, which is the decreased speech output.
4. Anhedonia, which can be seen as the reduced ability to express pleasure when exposed to positive stimuli.
5. Asociality, which is a decreased interest in socializing.

1.3 Treatment-resistant schizophrenia

About 40-60% of patients have exhibited responses to antipsychotic treatments. The rest are either unresponsive (10-30%) or partially responsive to treatments, where they show some improvement, especially in positive symptoms, but continue to have other mild to severe residual hallucinations, delusions, or more often, negative symptoms and cognitive impairment (Lehman et al., 2004).

Treatment resistance schizophrenia (TRS) is defined as unresponsive symptoms for at least two antipsychotic treatment trial with a sufficient dose and duration of not less than six weeks (Lehman et al., 2004; Potkin et al., 2020). In a recent systematic review, TRS treatment was shown to be an economic burden 3-11 folds higher than Non-TRS schizophrenia (Kennedy et al., 2014).

Limited options are available when antipsychotic mono-therapy has been optimized in terms of dose and duration compliance, while significant and intense residual symptoms persist (Lehman et al., 2004). Unfortunately, several augmentation plans proved to be ineffective except for clozapine which is known to

have a higher efficacy; thus, according to the latest American Psychiatric Association guideline, schizophrenia patients with insufficient response to antipsychotic therapy or those with suicidal ideation or behavior should be considered for a trial of clozapine (Keepers et al., 2020; Herbert Y. Meltzer, Alphs, et al., 2003).

1.4 Schizophrenia treatment

Schizophrenia treatment targets include curing or alleviating positive and negative symptoms, enhancing adaptive functioning and quality of life, as well as improving recovery from the weakening effects of the disease such as cognitive impairment and depression (Lehman et al., 2004). Psychotic symptoms were managed initially using reserpine and, more frequently, chlorpromazine, which can cause extrapyramidal symptoms (EPS). However, its antipsychotic effect was subsequently linked to its dopaminergic receptors blockage (DeBattista C., 2017). This led to the discovery of new generation antipsychotic medications called atypical antipsychotics because they made us realize that antipsychotic effect is not necessarily correlated with EPS at the clinically effective doses. Among atypical antipsychotics, clozapine has a unique efficacy in managing TRS (Gammon et al., 2021).

1.5 Clozapine response

Clozapine, a tricyclic dibenzodiazepine, is an atypical antipsychotic with a high affinity for dopamine (D1 and D4), 5-hydroxytryptamine (5-HT2), muscarinic, and α-adrenergic receptors, as well as a weak D2 receptor antagonist (Rey, 2018), as shown in Figure 1.1. Clozapine short binding with the D_2 receptor provokes pharmacological action (Seeman, 2002, 2014). However, insufficient evidence is available with regards to the role of D_1.

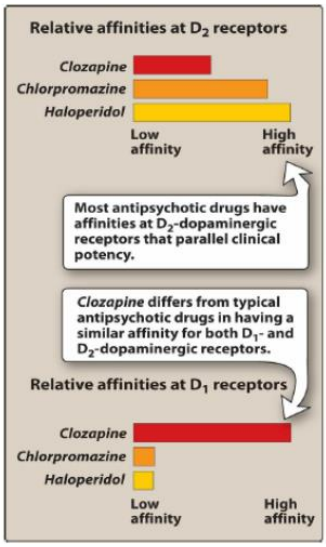

Figure 1.1 The relative affinity of clozapine to the dopaminergic receptors (Rey, 2018).

Following its discovery in 1959 and administration, 30 cases worldwide were reported to develop severe and fatal agranulocytosis (Griffith & Saameli, 1975; Idänpään-Heikkilä et al., 1975), which is a granulocyte count of fewer than 500 cells/mm^3 (Alvir et al., 1993). Consequently, clozapine was withdrawn by the manufacturer and was only used in some countries under the condition of weekly granulocytes monitoring (Hippius, 1999) until further results of clinical trials that revealed the superiority of clozapine over chlorpromazine in the treatment of schizophrenia as well as TRS (Claghorn et al., 1987; J. Kane et al., 1988). Since then, clozapine has been commonly used in treating schizophrenia, particularly in patients who are refractory or intolerant to the side effects of typical antipsychotics such as chlorpromazine and haloperidol (Howes et al., 2017).

Clozapine is the only second-generation antipsychotic drug approved to minimize the risk of suicide in patients with a history of schizophrenia (DeBattista C., 2017). Schizophrenia patients with a history of life-threatening suicide attempts should be critically evaluated for switching to clozapine. Any patient on a typical antipsychotic drug, risperidone or paliperidone with tardive dyskinesia, should be

switched to clozapine (DeBattista C., 2017). Clozapine is the first-line treatment for patients who are refractory to a minimum of two trials of antipsychotic drugs. In other words, it is the treatment of choice for unresponsive patients to two different types of antipsychotics with sufficient dose and duration (Silvia RJ et al., 2017). It is also the only drug proven to ameliorate the negative symptoms to some extent (Englisch & Zink, 2012). Recent findings from a meta-analysis of 68 articles revealed that clozapine treated SSD patients had lower hospitalization risk, all-cause discontinuation, and preferable outcomes in terms of clinical global impression severity and overall improved symptoms compared with other atypical antipsychotics, yet with unfavorable adverse effects like weight gain and type 2 diabetes (Masuda et al., 2019).

Population pharmacodynamic and pharmacokinetic (PKPD) model of clozapine in schizophrenia patients showed that the maximum improvement in the positive and negative symptoms scale (PANSS) is not more than 50% after 12 weeks of clozapine initiation (Shang et al., 2014). Dose recommendations were stratified based on sex and smoking status due to their significant effect on the pharmacokinetics of clozapine (Shang et al., 2014). Males and smokers were reported with lower clozapine concentration due to the higher activity of CYP1A2 among these groups, which is one of the most important metabolizing enzymes of clozapine (Haslemo et al., 2006; Jann et al., 1997)

1.6 Clozapine side effects

Compared to other antipsychotic drugs, clozapine has a lesser risk of undesired neurological effects (Englisch & Zink, 2012). Having an anticholinergic effect may help minimize the risk of EPS (Rey, 2018). However, only 5% of eligible

patients receive clozapine mainly due to its adverse effects, potentially delaying its initiation (Martini et al., 2021). The most serious adverse effects of clozapine are agranulocytosis which affects 1-2%, and neutropenia affecting more than 10% of the patients (DeBattista C., 2017; Rettenbacher et al., 2010; Rey, 2018; Silvia RJ et al., 2017). Due to these side effects, white blood cells and absolute neutrophils count (ANC) monitoring are recommended weekly after the initiation until six months, every other week until one year, and monthly after one year of clozapine initiation (Boazak et al., 2018). Weight gain is one of the other serious clozapine adverse effects affecting 30% of the patients, which together with hyperglycemia might lead to type 2 diabetes mellitus. Tachycardia, tremor, hypertension, orthostatic hypotension, abdominal pain, vomiting, constipation, diarrhea, nausea, agitation, akathisia, amnesia, blurred vision, confusion, delirium, convulsions, seizures, dizziness, delusion, dry mouth, fatigue, enuresis, headache, fever, heartburn, hypersalivation, hyperkinesia, insomnia, rash, restlessness, sleeplessness, syncope, sweating have also been reported as adverse effects of clozapine (Iqbal et al., 2020). A clozapine-induced seizure can be controlled by reducing the dose, as it was found to be a dose-dependent adverse effect. However, in general, clozapine's adverse effects are weakly correlated with its plasma concentrations (Pacia & Devinsky, 1994; Yusufi et al., 2007).

1.7 Clozapine pharmacokinetics

Following oral administration, clozapine undergoes fast and complete absorption with a time to maximum concentration (T_{max}) of 1.5–2 h. At around 4 h after administration, the maximum effect of the drug appears (Cheng et al., 1988; Fitton & Heel, 1990). Clozapine passes the blood-brain barrier and can be secreted in

breast milk. It is highly bounded by plasma proteins (around 95%), while approximately 50–73% undergoes first-pass metabolism (Cheng et al., 1988; Jann et al., 1993; G. Schaber et al., 1998). Demethylation, oxidation of the aromatic ring in the seven-positions and eight-positions, and conjugation are the major clozapine metabolic pathways. Clozapine is metabolized by CYP1A2 and CYP3A4 enzymes in the liver to form norclozapine or N-desmethylclozapine, which is considered the major metabolite (20–30%) (Eiermann et al., 1997). Norclozapine is not only a strong 5-HT1C receptor antagonist but also has a similar affinity to clozapine for D2 and 5-HT2 receptors (Kuoppamäki et al., 1993). Flavin monooxygenase 3 (FMO3) mediates the N-oxidation of clozapine (~10% of metabolites) (Fang et al., 1998; Pirmohamed et al., 1995). 8-hydroxy- 8-dechloro-norclozapine and its glucuronide, 7-hydroxynorclozapine sulfate, and clozapine N-oxide are the main urinary components, while the amounts of clozapine, 7-hydroxy-clozapine glucuronide, and norclozapine are minor. Figure 1.2 is the illustration of the clozapine metabolic pathway.

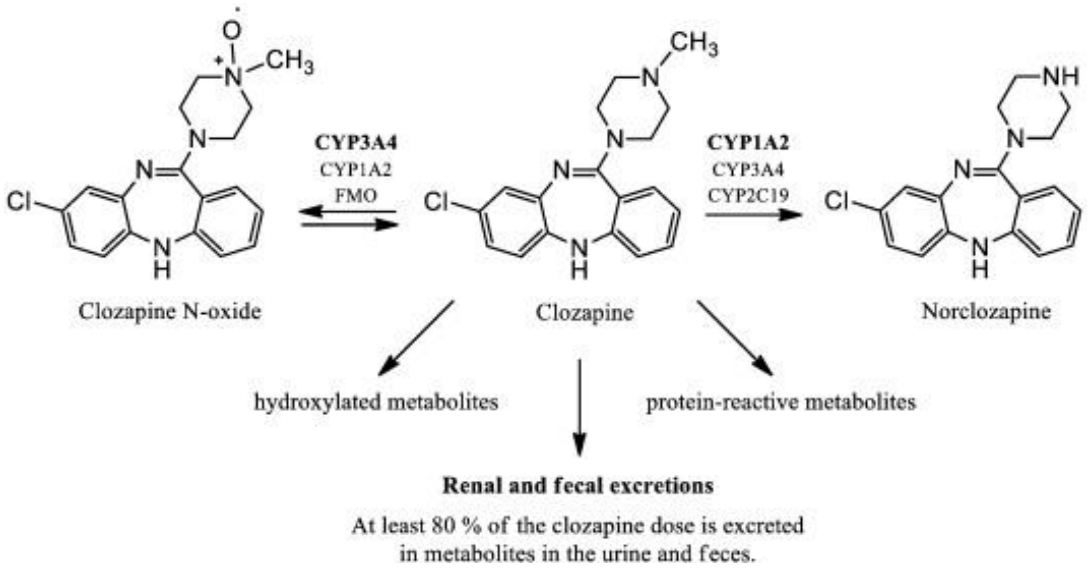

Figure 1.2 Clozapine metabolic pathway (Gonçalves et al., 2015)

Clozapine N-oxide is more frequently excreted in the urine than aromatic ring hydroxylation and conjugation products. However, it is not known whether any of these metabolites are of pharmacological or toxicological importance (Gisela Schaber et al., 2001). It has been assumed that the free fractions of clozapine, norclozapine, and clozapine N-oxide are filtered through the kidney glomerulus. About 90% of the filtered clozapine undergoes tubular reabsorption. However, norclozapine and clozapine N-oxide are assumed to be secreted by the tubule. The elimination half-life (T1/2) of clozapine was reported to be 8 hours after a single dose. Longer half-lives between 14 and 17 hours have been reported in steady-state dosing (G. Schaber et al., 1998).

1.8 Clozapine and norclozapine therapeutic drug monitoring

Based on the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry, clozapine therapeutic drug levels should be carried out in the cases of dose optimization, patient's non-compliance, non-responsiveness, relapse, adverse effects, children and adolescence, elderlies, and pharmacokinetic comorbidities (liver disease, suspected drug-drug interactions, genetic polymorphism) (Hiemke et al., 2011).

The sample should be taken at the steady-state, preferably before the morning dose, and typically, multiple measurements are required to establish an appropriate recommendation, e.g., a single measurement might not sufficiently identify the reason behind a subtherapeutic level whether, it is due to rapid elimination, poor bioavailability, or non-compliance (Hiemke et al., 2011).

According to AGNP, chromatographic techniques such as high-performance liquid chromatography (HPLC), in combination with appropriate detection methods, are highly recommended for monitoring clozapine as well as its primary metabolite (norclozapine). The therapeutic range of clozapine was reported to be 350-600 ng/mL. Chromatographic techniques are adequately robust, accurate, and precise. A primary shortcoming of these methods is that they require certain sample preparation before chromatographic analysis, limiting the sample throughput (Hiemke et al., 2011). Sample preparation was the focus of extensive research in analytical chemistry, and the traditional liquid-liquid or solid-phase extraction methods underwent further updates to produce simple, practical, safe, and economically acceptable newer methods with an improved lower limit of quantification (LLOQ) (Niu et al., 2018).

1.9 Pharmacometrics

According to the Food and Drug Administration (FDA), pharmacometrics is defined as the science that quantifies medication, disease, and trial information to assist in drug development and/or regulatory decisions. Pharmacometrics models may describe the relationship between drug pharmacokinetics as a measure of exposure, pharmacodynamics (or response) for both wanted and unwanted effects, and individual patient characteristics. Disease models describe the relationship between biomarkers and clinical outcomes, the time course of the disease, and placebo effects. The trial models describe the inclusion/exclusion criteria, compliance, and patient discontinuation. The standard scope of pharmacometrics has been the drug models, also referred to by terms such as dose-response, concentration-effect, PKPD relationships. These pharmacometrics models are performed in the

context of drug development, therapeutic, and regulatory decisions. The ability to integrate knowledge across the development program and compounds and biology is the most significant advantage of these analyses (*Division of Pharmacometrics | FDA*, 2018).

1.9.1 Population pharmacokinetic models

Population pharmacokinetics is the investigation of drug pharmacokinetics in terms of population level. The data obtained from every subject in a population are analyzed together through a nonlinear mixed-effects model. “Nonlinear” means that the dependent variable such as the concentration is nonlinearly related to the independent variables such as the time and dose as well as other model parameters. This relationship is described in the structural model. “Mixed-effects” means the combined implementation of the “fixed” and “random” effects, where fixed effects are the constant population parameters across individuals and random effects are the varying parameters between individuals that are described in the statistical model. The statistical model accounts for both between-subjects variability as well as the residual error, which is the remaining unexplained variability between the individual prediction and the observation. After developing a structural and statistical model, a covariate model can be performed to evaluate predictors that might explain the variability of the population parameters across individuals (Mould & Upton, 2013). Pharmacokinetics data can be generally analyzed using the following approaches:

1.9.1(a) Non-compartmental analysis

The non-compartmental approach makes no assumption with regards to the drug pharmacokinetics in single or multiple compartments in the body. One example is the calculation of the area under the concentration-time curve (AUC) from the concentration time-points of the drug using the trapezoidal method (Rowland &

Tozer, 2011). The non-compartmental analysis is easy and less time-consuming. However, it requires extensive and homogeneous samples to be taken from all subjects. Furthermore, it is not possible to assess the covariate effects on pharmacokinetic parameters (Damoiseaux et al., 2022).

1.9.1(b) Compartmental analysis

The compartmental analysis involves the assumption of multiple or single compartments where the drug is distributed immediately and evenly (Rowland & Tozer, 2011). Compartmental modeling does not require intensive or homogeneous sampling, thereby enhancing the precision of parameters' estimates and empowering the model to detect covariates' effects. Compartmental modeling provides a better mechanistic insight into a drug's underlying pharmacokinetic processes and can help make dose adjustments (Damoiseaux et al., 2022). On the other hand, the compartmental model does not usually represent any physiological composition; instead, the compartments function as a scheme for the estimation of the pharmacokinetic parameters (H. Jones & Rowland-Yeo, 2013).

1.9.1(c) Physiological-based pharmacokinetic models

Physiological-based pharmacokinetic models (PBPK) are developed based on known physiological structures and therefore are complex and consist of many compartments representing the different tissues or organs connected by flow rates parallel to the blood circulation. The major advantage of PBPK models is that they can include biochemical and physiological variability sources in the model parameters. Furthermore, a simulation can be performed to generate data representing physiological and anatomical variables. The ability to evaluate variability in a population is specifically crucial in the case of drug-drug interactions

(H. Jones & Rowland-Yeo, 2013). One example is the PBPK model to describe the pharmacokinetics of ciprofloxacin (J. F. Schlender et al., 2018).

1.9.1(d) Semi-physiological pharmacokinetic models

Semi-physiological pharmacokinetic models are less complex than physiologically based pharmacokinetic models and involve concepts from compartmental population pharmacokinetic models as well as a representation of a physiological process of interest, such as the pre-systematic drug metabolism addressed previously in the case of sunitinib (Yu et al., 2015) and dextromethorphan (Abduljalil et al., 2010) and will be here employed to account for the pre-systemic metabolism of clozapine, which undergoes a significant hepatic first-pass metabolism of 50–73% (Cheng et al., 1988; Fitton & Heel, 1990; Jann et al., 1993; G. Schaber et al., 1998).

1.9.2 Time-to-event models

The time-to-event or survival model is defined as the method for evaluating the time length until a pre-defined outcome or event of interest occurs. One of the main aspects of survival data is that part of the individuals might not experience the event of interest, such as death, by the end of the follow-up, which means that those individuals' survival time is unknown. We refer to this case as censoring (Schober & Vetter, 2018). Time-to-event analysis can be used instead of regular pharmacodynamic models to account for binary categorical drug response such as graft survival and investigate the relationship with drug exposure in terms of concentration or dose using different parametrization of maximum effects models (Frobel et al., 2013).

Several approaches were proposed to analyze the time-to-event data, i.e., parametric, semi-parametric, and non-parametric methods. Non-parametric

approaches make no hazard distribution assumptions, such as the Kaplan-Meier estimator and log-rank test. Furthermore, they qualitatively identify the categorical covariates. Semi-parametric methods, such as cox-regression, quantitatively evaluate the covariate effect on hazard ratio with no assumptions on hazard distribution and a tendency to overparameterize with a limited sample size (Bursac et al., 2008; Pavlou et al., 2015; Vittinghoff et al., 2012). Parametric method using nonlinear mixed-effects time-to-event modeling is known to be practical and highly effective where a specific survival time distribution is considered (Schober & Vetter, 2018). The parametric method completely specifies hazard function and can evaluate any type of covariates, including the time-varying ones and the covariates that may influence each other. Moreover, this approach considers censoring and simulation of time-to-event data depending on the final model (Frobel et al., 2013; Sheikh Ghadzi et al., 2018). In the current study, the parametric time-to-event model was used to describe the responsiveness of clozapine taking into account the time factor and based on the absence of positive symptoms in schizophrenia patients.

1.9.3 Disease progression models

Disease progression models are mathematical descriptions of the disease status as a function of time mainly employed to better account for the drug effect in the longitudinal data obtained from clinical trials and observational studies (Mould et al., 2007; Venuto et al., 2016). Furthermore, predictors for both baseline disease status and disease progression can be investigated (Passey et al., 2015; Samtani et al., 2013). Disease progression is a powerful tool used to support drug development by quantifying the magnitude and shape of both the disease progression process and the drug effect. In addition, the principle of disease progression models can also be implemented to describe other continuous parameters in the disease, for example the

adverse events of clozapine. In the current work, the disease progression model was used to describe the changes of pulse rate, body mass index, and absolute neutrophil count over time. The disease progression model can be classified into three major types (Cook & Bies, 2016), as summarized below.

1.9.3(a) Empirical models

Empirical models purely describe the data rather than the underlying biological mechanism. The advantage of empirical models is, in general, the simplicity and the direct description of the linear or nonlinear disease biomarker time curve. The empirical models have been adapted to describe disease status in many areas, especially when the disease severity, e.g., a scoring system is monitored with consistent time intervals, such as in schizophrenia, depression, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease. In the present study, principles from the empirical disease progression models were employed to account for the time course of the adverse effects reactions of clozapine in schizophrenia patients.

1.9.3(b) Systems biology models.

Systems biology models are detailed mathematical descriptions of the molecular mechanisms in pathophysiology, pharmacology, and biology. In general, to develop systems biology models, an extensive scientific collaboration should be carried out and integration of results from clinical, non-clinical, in vivo, ex vivo, and in vitro trials. For example, calcium homeostasis and bone remodeling were described in a system biology model (Peterson & Riggs, 2012).

1.9.3(c) Semi-mechanistic models

Semi-mechanistic models take into consideration both the data and the underlying biological process. As opposed to systems biology models, semi-mechanistic models do not involve the representations of the detailed molecular mechanisms. Instead, a reduced mathematical description of the most important biological processes with average complexity between the empirical and systems biology models to represent the data adequately. One example would be the bone mineral density and bone turnover model investigating osteoporosis therapies effects (van Schaick et al., 2015).

1.9.4 Mixture models

In principle, parametric modeling involves the assumption of the symmetric distribution of inter-individual variability (IIV) and residual variability around the population parameter estimates. However, this is not always the case, so certain steps should be taken to ensure that the data does not violate that assumption, e.g., the transformation of the skewed distribution or mixture models for the bi-modal distribution (Carlsson et al., 2009).

Since its development and implementation in nonlinear mixed-effect modeling (NONMEM) by Beal and Shiner (Beal et al., 2008), the mixture model was used in many creative applications to account for the multimodal distribution of pharmacokinetic and pharmacodynamic parameters. An example of mixture model applications is modeling responders and non-responders in clinical trials with dose escalation design. In this case, the distribution of drug response does not show a symmetrical shape, meaning that non-responders represented a separate sub-population with an underlying reason of non-responsiveness besides the drug dose or concentration (Shiiki et al., 2002). The current approach to classifying drug response

(and side effects) is based on cutoff criteria limiting its accuracy and can be enhanced by changing to data-driven alternatives for classification such as using the mixture models (Bouguila & Fan, 2019). Mixture models capture data heterogeneity by dividing the population into subgroups, each of which is defined by a certain set of parameters (Cole & Bauer, 2016). In the current study, the application of the mixture model to describe the susceptibility of the patients to the adverse effects of clozapine was explored.

1.10 Problem statement and study rationale

Therapeutic drug monitoring (TDM) of clozapine is clinically relevant in certain situations, such as inadequate clinical response, signs of toxicity, the onset of seizures, changes in concurrent medications, concurrent use of caffeine or smoking, concomitant liver disease, and suspected non-compliance (Greenwood-Smith et al., 2003). However, routine TDM of clozapine is still limited, particularly in Malaysia. Furthermore, the analytical methods available for the measurement of plasma concentrations of clozapine and norclozapine require a large sample volume and a long preparation time. Therefore, this study was designed to develop a new analytical procedure for the determination of clozapine and norclozapine concentrations in plasma with a short preparation time. The newly developed analytical method can be further implemented as the routine TDM of clozapine in the pharmacy practice in Malaysia.

Clozapine has significant unexplained inter- and intra-individual variations in plasma concentrations and complex metabolism (Spina et al., 2000), where 50–73% undergoes pre-systemic biotransformation (Cheng et al., 1988; Jann et al., 1993; G. Schaber et al., 1998). However, clozapine’s pre-systemic metabolism was

inadequately addressed in population pharmacokinetic models available in the literature. Factors affecting the clozapine plasma concentrations vary significantly from study to study, and predictors of the variability are inconclusive. According to Perry's dosing nomogram, 47% of clozapine concentration variabilities were explained by dose, sex, and smoking status (Perry et al., 1998), while dose, sex, cigarette smoking, body weight, clozapine concentration, and clozapine:norclozapine ratio, accounted to only 48% of the clozapine concentration variabilities in Rostami-Hodjegan nomogram (Rostami-Hodjegan et al., 2004). Unexplained variability in clozapine pharmacokinetics may be explained by incorporating potential covariates such as the race and drug-drug interactions. Population pharmacokinetics modeling is a robust tool for obtaining valuable drug pharmacokinetics information from both sparsely and intensively sampled data.

Pantoprazole is one of the most commonly prescribed proton pump inhibitors (PPIs) in Malaysia (Elnaem et al., 2017) and is commonly prescribed in psychiatric patients (Shuman et al., 2014). Pantoprazole was shown to have minimal interactions with other drugs because of a lower affinity for cytochrome P450 than older PPIs (Calabrese et al., 2007). However, its interaction with clozapine was not adequately assessed. This study was designed to address the research gaps mentioned above in the healthy volunteers population. Studies involving healthy volunteers are commonly used to assess the pharmacokinetics of a drug and quantify the factors affecting the elimination and metabolism, such as drug-drug interactions (Karakunnel et al., 2018) with higher accuracy after eliminating other comorbidities usually exists in patients. However, extensions of these models in the patients population should be developed for proper predictions and dosing adjustments (Karakunnel et al., 2018).

In terms of drug response, a maximum effect of 50% improvement in the PANSS was reported in a published PKPD model. However, the study duration was short (12 weeks) and did not take into account the time factor because PKPD models, generally, only involve the relationship between exposure and response. Clozapine treatment was recommended to be continued for not less than three months after achieving therapeutic range before patients can be declared as non-responsive in the latest consensus guideline for reporting and determining sufficient treatment response, which was introduced by the Treatment Response and Resistance in Psychosis (TRRIP) Working Group (Howes et al., 2017). The time factor can be accounted for in a time-to-event analysis, which allows the evaluation of time-varying covariates such as the drug exposure, taking into account censoring as well as the ability to perform simulations of time-to-event data (Frobel et al., 2013; Sheikh Ghadzi et al., 2018). The time-to-event analysis that is implemented in this study will give a better and more accurate quantification of the improvements of positive symptoms after the initiation of clozapine therapy by incorporating the time-varying covariates.

Clozapine is an underutilized drug mainly due to concerns regarding its potential adverse effects (Iqbal et al., 2020). Adverse effects are not only associated with dose delays, dose reductions, treatment cessation, low treatment adherence, and more extended hospitalization but also can be life-threatening (de Vries Schultink et al., 2016; Iqbal et al., 2020). Prediction and avoidance of adverse drug reactions (ADRs) have become a significant interest of personalized medicine to optimize drug therapies (Katzung, 2017). By utilizing the disease progression and mixture models, the time course and individual susceptibility to adverse drug effects such as the ones

related to clozapine can be described in a novel approach in pharmacometrics as what was portrayed by this study.

Based on the above explanations, pharmacometrics models of clozapine developed in the current study may shed light on the monitoring of clozapine efficacy, safety, and interaction.

1.11 Aim and objectives

1.11.1 General objective

The objectives of the current study were to develop a new analytical method of measuring clozapine and norclozapine as well as to develop and validate pharmacometrics-based models for clozapine and norclozapine pharmacokinetics and pharmacodynamics in healthy volunteers and SSD patients.

1.11.2 Specific objectives

1. To develop and validate a new analytical procedure for determining clozapine and norclozapine concentrations in the human plasma using HPLC.
2. To develop and validate population pharmacokinetic model for clozapine and norclozapine in healthy volunteers with covariates effects (e.g., age, gender, body mass index (BMI)).
3. To investigate the pharmacokinetic interaction between clozapine and pantoprazole.
4. To develop and validate a repeated time-to-event model for the improvement of positive symptoms following the initiation of clozapine treatment in SSD patients with covariates effects, including the drug dose as a measure of exposure.

5. To quantify the development of adverse drug effects related to ANC drop, weight gain, and tachycardia in SSD patients receiving clozapine by incorporating a novel use of disease progression and mixture models.

CHAPTER 2

LITERATURE REVIEW

2.1 Clozapine and norclozapine analysis

2.1.1 Clozapine and norclozapine analysis in literature

Clozapine and norclozapine analyses were performed most commonly using HPLC (Hiemke et al., 2011), while in only a few studies, gas chromatography (Bondesson & Lindstrom, 1988) and capillary electrophoresis (Ho et al., 2004) were used. Most of the HPLC analyses have utilized a nonpolar stationary phase of octadecyl silanol (C-18), while few have used hexyl silanol (C-6) (Avenoso et al., 1998), C-6 phenyl (Rosland et al., 2007), or octyl silanol (C-8) (Åkerman, 1997; Mercolini et al., 2007; Raggi et al., 1999) with a polar mobile phase consisting of a mixture of acetonitrile and/or methanol with either ammonium acetate or phosphate buffer and/or triethylamine. Ultraviolet (UV) absorption detection was used in most of the studies found in literature, while mass spectroscopy (MS) (Aravagiri & Marder, 2001; Choong et al., 2009; Kollroser & Schober, 2002; Niederländer et al., 2006) and amperometric (AM) (Raggi et al., 1999, 2000) detections were used in some studies in an attempt to achieve higher sensitivity and selectivity. However, both AM and MS are often troublesome to be used due to the complexity of instrumentation.

2.1.2 Extraction methods

The drug and metabolites are usually diluted in the plasma, which consists of a complex structure, thus requiring a cleanup step prior to the HPLC analysis in order to purify the sample of these interfering components. The cleanup process is also required to concentrate the drugs and the metabolites to an appropriate concentration for detection and quantification purposes (Behbahani et al., 2013).

Liquid-liquid extraction (LLE) is one of the earliest techniques used in the sample clean-up process. LLE usually requires a large volume of the sample as well as large amounts of toxic and expensive extraction solvents, in addition to being time-consuming. Solid-phase extraction (SPE) is faster and employs less toxic solvents; however, it is expensive and requires column conditioning that may not be practical for routine TDM (Behbahani et al., 2013). Thus, developing and optimizing of a new extraction technique is warranted to achieve a faster, safer, and less expensive procedure (Molaei et al., 2015).

This led to the invention of solid-phase microextraction (SPME) using small amounts of extraction phase loaded on a fiber or film instead of the column (Psillakis & Kalogerakis, 2003). Several attempts were reported in the literature to improve the extraction rate, sensitivity, and reusability of the film for the SPME. However, the fibers or films required in this method are expensive with a short lifetime, plus the variability from lot to lot in length and thickness of coating might result in a variable extraction ratio (Psillakis & Kalogerakis, 2003).

In addition to SPME, attempts were initiated to upgrade the LLE by minimizing the amounts of extraction solvents that led to the development of a so-called liquid-phase microextraction (LPME) (Psillakis & Kalogerakis, 2003). Improvement on the LPME method was presented by adding a dispersive solvent, which is relatively miscible in both phases and thus reduces the interfacial tension, increases the surface contact area, and improves mass transfer (Behbahani et al., 2013). This method was called dispersive liquid-liquid microextraction (DLLME). However, this method requires large amounts of dispersive phase, which is usually chlorinated toxic solvents (Molaei et al., 2015).