Pharmacokinetic Study of Tramadol Rectal Suppository in Healthy Volunteers

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ABSTRACT

Rectal administration of tramadol can serve as a good alternative in postoperative pain, or situations where oral administration is not applicable. Tramadol is available only as a capsule for oral use and as a solution for injection in Thailand. Clinical uses and manufacturing of tramadol suppository are promising, while pharmacokinetic of rectal suppository has not been investigated. Thus, the main objective of this study is to examine pharmacokinetic parameters of tramadol after rectal administration. Conventional form of tramadol rectal suppository was formulated in polyethylene glycol (PEG) base and in vitro evaluation was performed. A single dose of tramadol rectal suppository 100 mg was administered to healthy volunteers (n=14). Blood samples were collected at pre-determined time interval (0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours). A plasma concentration of tramadol and metabolites, *O*-desmethyltramadol (ODT) and *N*-desmethyltramadol (NDT) were determined by a HPLC method with fluorescence detection (λ excitation 202 nm, λemission 310 nm). Sample preparation involves ethyl acetate extraction. The compounds were separated using C18 column and eluted by mobile phase consisting of acetonitrile-0.05M phosphate buffer, pH 7 (20:80) at 30℃ (1.0 mL/min). Methocarbamol was used as an internal standard. The compounds were well separated and required 20 min run time for each analysis. The analytical method was validated accordingly: LLOQ of tramadol (14.0 ng/mL), ODT (5.48 ng/mL), NDT (7.71 ng/mL); recovery of tramadol and ODT was higher than 75%, NDT higher than 65% and methocarbamol 60.3%; accuracy and precision within ±15% for all compounds

and stability was shown by percent remaining of tramadol (102.1%), ODT (101.4%) and NDT (99.5%) after 12 hours at 37ºC revealing no degradation at experimental condition.

Pharmacokinetic parameters were analyzed by the non-compartmental method using PK-solver. The pharmacokinetic parameters, i.e. C_{max}, T_{max}, $AUC_{0-\infty}$ and $T_{1/2}$ for tramadol, ODT and NDT were 349.96 ± 80.4 ng/mL, 66.92 ± 30.2 ng/mL, 22.97 ± 18.3 ng/mL; 3.93 ± 0.7 h, 6.07 ± 1.5 h, 6.93 ± 1.7 h; 4.69 ± 1.5 μg.mL/h, 1.09 ± 0.41 μg.mL/h, 0.40 ± 0.4 μg.ml/h; 6.59 ± 1.4 h, 8.28 ± 4.5 h, 7.38 ± 3.0 h, respectively. Parameters such as C_{max}, AUC and $t_{1/2}$ were similar to those reported values from rectal and oral dosage forms. Longer T_{max} of tramadol, ODT, NDT compared to oral IR tablets implied delayed absorption of rectal administration. This value, however, agreed with reported value of oral SR tablets. In conclusion, pharmacokinetic of tramadol following rectal administration has been demonstrated, which comparable pharmacokinetic profiles to those from oral dosage forms was revealed.

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LIST OF ABBREVIATIONS AND SYMBOLS

º C	Degree celsius
µg	microgram
µL	microliter
5-HT	5-hydroxytryptamine/Serotonin
AUC	Area under the plasma concentration time curve
C_{max}	Maximum plasma concentration
Cl/F	Clearance
CNS	Central nervous system
FDA	Food and Drug Administration
Fig	Figure
GC	Gas Chromatography
GI	Gastrointestinal
h	Hour
HPLC	High Performance Liquid Chromatography
HCl	Hydrochloride
IM	Intra-muscular
IR	Immediate release
IS	Internal Standard
k_e	Elimination rate constant
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
M	Mole
MAO	Monoamine oxidase
mL	milliliter
MRT	Mean Residence Time
MS	Mass spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

NDT	*N*-desmethyltramadol
NE	Norepinephrine
ng	nanogram
ODT	*O*-desmthyltramadol
PEG	Polyethylene glycol
QC	Quality control
RSD	Relative standard deviation
SC	Subcutaneous
SD	Standard deviation
SNRI	Serotonin-norepinephrine re-uptake inhibitor
SR	Sustained release
T	Tramadol
$T_{1/2}$	Half-life
T_{max}	Time to maximum plasma concentration
Tab	Table
v/v	Volume by volume
V_z/F	Volume of distribution

Chapter 1

INTRODUCTION

1.1 General Introduction

Tramadol hydrochloride (tramadol), (1*RS*, 2*RS*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a synthetic centrally acting analgesic (Zwaveling *et al.*, 2004). It is a μ-receptor agonist, whereas inhibition of reuptake of norepinephrine and serotonin contribute to the analgesic effect (Zwaveling *et al.*, 2004). Tramadol is used for moderate to severe pain associated with trauma, renal or biliary colic pain, labor or chronic pain either of malignant or non-malignant origin (Zwaveling *et al.*, 2004).

1.1.1 Pharmacodynamics

Tramadol possess a modest affinity to μ-opioid receptors and no affinity to δ or k receptors. Tramadol affinity to μ-receptor is about 10 times weaker than codeine, 60 times weaker than dextropropoxyphene and 6000 times weaker than morphine (Leepert, 2009). However, *O*-desmethyltramadol (M1) exhibits 300 fold greater affinity to μ-receptor than parent compound. Tramadol also exerts the analgesic activity via pain descending inhibitory system, which involved norepinephrine (NE) and serotonin (5HT) (Leepert, 2009). Activation of the inhibitory neurons relates to release of NE and 5HT, and thus blockage of pain transmission (Steeds, 2009).

Tramadol is a racemate mixture of (+) and (-) tramadol. (+) - Tramadol and its (+) - M1 metabolite showed 2-fold and 700 fold greater affinity to μ-receptor respectively, than (±) tramadol (Table 1). Additionally, (+) tramadol is 4 fold more potent than (-) tramadol in inhibiting 5HT reuptake while (-) tramadol is approximately 10 times more potent than (+) tramadol in inhibiting NE uptake (Table 1) (Grond and Sablotzki, 2004, Leepert, 2009). Thus, tramadol action appeared to be stereoselective

and multimodal, which (+) tramadol inhibits 5HT reuptake, (+) M1 acts as opioid agonist, and (-) tramadol inhibits NE reuptake.

Table 1 Relative activity for inhibition of opioid receptor binding or monoamine uptake (Grond and Sablotzki, 2004)

Drug	Affinity for μ opioid receptor [K_i (μmol/L)	Uptake inhibition	
		NE	5HT
(±) Tramadol	2.1	0.78	0.9
(+) Tramadol	1.3	2.51	0.53
(-) Tramadol	24.8	0.43	2.35
(+) M1	0.0034		
Morphine	0.00034	IA	IA
Imipramine	3.7	0.0066	0.021

IA: Inactive, Ki : Inhibition constant

Tramadol provides analgesia in acute pain comparable to some opioid and non-opioid analgesics (Grond and Sablotzki, 2004). It had been demonstrated that tramadol 300-600 mg/day and morphine 10-60 mg/day yield similar response in cancer patients (Grond and Sablotzki, 2004). Due to its minimal effect on respiratory function, which is an important advantage over morphine, tramadol can be a good alternative for labour pain, traumatic pain, as well as those with increased risk of respiratory dysfunction (i.e. elderly, smokers). Besides, children and pre-existing cardiopulmonary disease are expected to benefit from this property (Grond and Sablotzki, 2004). Moreover, constipation, neuropsychiatric symptoms, and dependence are less in tramadol compared to morphine. It is also showing fewer adverse gastrointestinal effects (Grond and Sablotzki, 2004).

1.1.2 Pharmacokinetics

Absorption

Tramadol can be administered either orally or non-orally, i.e. subcutaneous (SC), intravenous (IV), intramuscular (IM), and rectal. Peak plasma concentration (C_{max}) attained after administration of oral drops and capsules were 1.2 and 1.6-1.9 hours (Grond and Sablotzki, 2004). Following single oral dose of 100 mg, C_{max} is approximately 300 ng/mL (Grond and Sablotzki, 2004), but it can vary from 100-300 ng/mL. Oral bioavailability is 70% in healthy volunteers following single dose. Following multiple oral administration, the increase in bioavailability, $C_{max,}$ and AUC observed was partly due to the saturated first pass metabolism (Grond and Sablotzki, 2004). Sustained release oral formulation shows absolute bioavailability of 67.3% relative to intravenous formulation, C_{max} of 141.7 ± 40.4 µg/L and T_{max} of 4.9 ± 0.8 hours (Grond and Sablotzki, 2004). Absorption pharmacokinetic of tramadol after various dosage forms is summarized in Table 2.

Table 2 Pharmacokinetic parameters of tramadol in various dosage forms (tramadol 100 mg in each dosage form**)**

Dosage form	C_{max} (ng/mL)	T_{max} (h)	AUC (µg.h/mL)	$T_{1/2}$ (h)	References
SR tablet	215.5	5.5	2.6	6.1	Gu and Fawcett, 2005
Tablets	337.4 ± 60.8*	1.5 ± 0.5*	2.61 ± 0.53*	7.0 ± 1*	Ardakani and Rouini, 2007
	314.4 ± 53.3**	1.8 ± 0.4**	3.02 ± 0.7**	7.1 ± 0.4**	
Tablets	170.4 ± 44.5	1.8 ± 0.4	1.4 ± 0.5	5.2 ± 0.9	Rouini *et al.*, 2006
Rectal suppository	293.6 ± 50	3.29 ± 1.29	2.9 ± 0.3	5.72 ± 1.04	Lintz *et al*., 1998

*Male, **Female

After rectal administration of 100 mg suppositories, tramadol absorption begins within a few minutes (0-22 min) (Grond and Sablotzki, 2004). A C_{max} of 294 ng/mL was reported in 3.3 hours (Grond and Sablotzki, 2004). Similar T_{max} of 2.5 hours was reported ((Nobilis *et al.*, 2002). Bioavailability is 77% for rectal suppository (Mercadante *et al.*, 2005). The onset of absorption, increase in T_{max} as well as increase in bioavailability of rectal compared to oral administration can be explained with anatomical description of rectum.

Distribution

Tramadol is rapidly distributed in the body with plasma protein binding approximately 20% (Grond and Sablotzki, 2004). Volume of distribution after oral and IV administration in young male healthy volunteers were 306 L and 203 L, indicating high tissue affinity of tramadol (Grond and Sablotzki, 2004, Leepert, 2009). In a rodent model, tramadol was particularly distributed into the lungs, spleen, liver, kidneys and brain (Grond and Sablotzki, 2004). Tramadol passes the placental barrier; the concentration in umbilical veins is reported 80% of the concentration in the mother's vein (Grond and Sablotzki, 2004, Leepert, 2009). Very small amounts (0.1%) of tramadol and ODT are excreted in breast milk, and have been detected within 16 h after administration (Grond and Sablotzki, 2004).

Metabolism

Tramadol undergoes biotransformation in the liver, mainly by the phase I *O*- and *N*-demethylation, followed by conjugation of the demethylated compounds (Grond and Sablotzki, 2004). Cytochrome P 2D6, 2B6, and 3A4 are involved in demethylation (Subramanyam *et al.*, 2001, Rouini *et al.*, 2006). Eleven metabolites are produced in first phase reactions in which *O*-desmethyltramadol (ODT) is a major metabolite (Figure 1) (Leepert, 2009). The main metabolites of tramadol, M1 and mono-*N*-desmethyltramadol (NDT or M2) are further metabolized to three additional secondary

metabolites namely *N,N*-didesmethyltramadol (M3), *N,N,O*-tridesmethyltramadol (M4) and *N,O*-desmethyltramadol (M5) (Campanero *et al.*, 2004). All metabolites are further conjugated with glucuronic acid and sulphate before excretion in urine (Campanero *et al.*, 2004). Metabolic pathway of tramadol was shown to be stereoselective (Campanero *et al.*, 2004). Rouini *et al*. showed that after oral administration of 100 mg tramadol, C_{max} of tramadol, ODT and NDT were 170.4 ± 44.5 ng/mL, 51.6 ± 5.7 ng/mL and 35.5 ± 4.0 ng/mL, respectively (Rouini *et al.*, 2006). Similar concentration time profile of tramadol was obtained after rectal administration, in which C_{max} of tramadol and M1 of 796 nmol/L (209.66 ng/mL) and 214 nmol/L (53.37 ng/mL), respectively, were revealed (Nobilis *et al.*, 2002). After oral or rectal administration of tramadol 100 mg, the t_{max} of M1 is about 1.4 hours longer than that of tramadol and C_{max} of ODT is no more than 18-26% of that tramadol (Grond and Sablotzki, 2004).

Elimination

Approximately 90% of the drug is excreted by the kidneys and 10% is excreted in feces (Leepert, 2009). The elimination half-life is approximately 5-6 h for tramadol and approximately 8 h for ODT (Leepert, 2009). All metabolites are almost completely excreted via the kidney. In renal impairment (creatinine clearance < 79 mL/min), decreased excretion of tramadol and ODT, compared to healthy individuals with normal renal function (creatinine clearance >100 mL/min), was reported (Leepert, 2009). In patients with advanced cirrhosis, there was a decrease in tramadol metabolism with a concomitant decrease in hepatic clearance and increase in blood serum levels. In these patients, the observed elimination half-life is 2.5 times longer than normal values (Leepert, 2009).

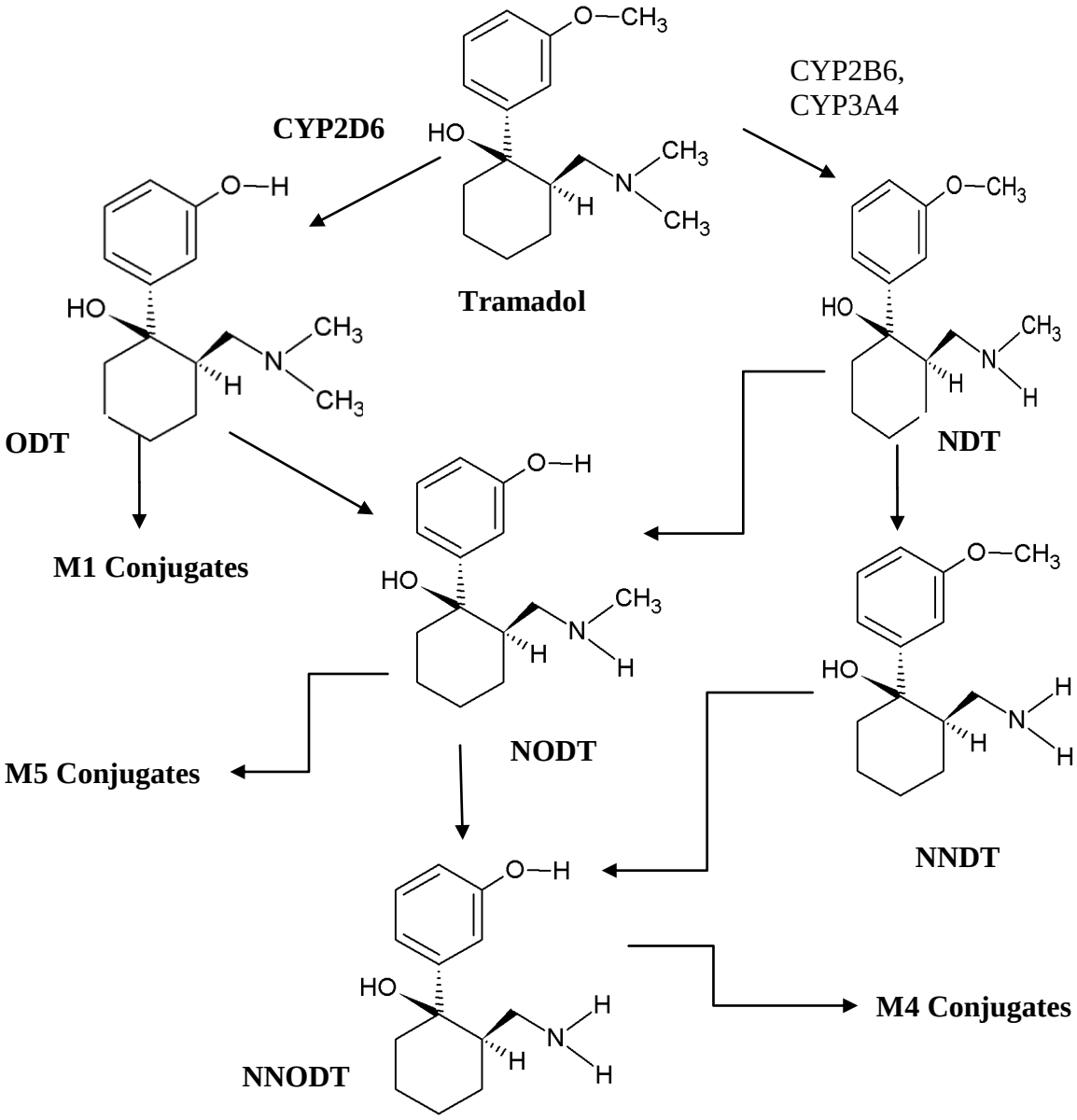

Figure 1 Metabolic pathway of tramadol (Subramanyam *et al.*, 2001)

1.1.3 Adverse effects

At recommended dosages, tramadol is generally well tolerated. The frequency of some adverse effects may be related to dose and route of administration. The most common adverse effects were central nervous system (e.g. dizziness) and GI disturbances. The incidence of adverse effects of tramadol is shown in Table 3.

Table 3 Adverse effects of tramadol

GI	Incidence (%)	**CNS**	Incidence (%)
Constipation	24-46	Dizziness	26-33
Nausea	24-40	Vertigo	26-33
Vomiting	9-17	Sedation	16-25
Diarrhea	5-10	Headache	18-32
Dyspepsia	5-13	CNS stimulation	7-14
Dry mouth	5-10	Asthenia	7-12
Abdominal pain	1-5	Hallucination	<1
Anorexia	1-5	**Genitourinary**	
Dermatological		Urinary frequency	1-5
Itching/Pruritis	8-11	Urinary retention	1-5
Sweating	6-9	Menopausal symptom	1-5
Rash	1-5	Menstrual disorder	<1
Urticaria	<1	**Cardiovascular**	
		Vasodilation	1-5
		Hypotension	<1

Tramadol is extensively metabolized by a number of pathways including CYP2D6 and CYP3A4. The formation of ODT is dependent upon CYP2D6 and as such is subjected to inhibition, which may affect the therapeutic response. Therefore, co-administration of tramadol with a CYP2D6 inhibitor (e.g., fluoxetine, paroxetine, quinidine) may increase concentration of tramadol and reduce concentrations of M1. Co-administration of tramadol with CYP3A4 inhibitors (e.g., azole antifungals, macrolide antibiotics, protease inhibitors) may decrease tramadol clearance, and CYP3A4 inducers (e.g., carbamazepine, phenytoin, rifampin) may increase tramadol clearance (Drug Facts and Comparison, 2013).

Potential drug-drug interaction from concurrent drug treatment was reported as follows:

- Drugs associated with serotonin syndrome

Serotonin syndrome has been reported during post-marketing experience in patients receiving tramadol concomitantly with MAO inhibitors, SSRIs, SNRIs, or α_2-adrenergic blocking agents. Tramadol decreases the synaptic reuptake of the monoamine neurotransmitters norepinephrine and serotonin, and animal studies have shown increased deaths with combined administration of tramadol and MAO inhibitors. Therefore, tramadol should be used with great caution in patients receiving other drugs that may affect serotonergic neurotransmission, including MAO inhibitors, SSRIs, triptans, linezolid, lithium.

- Warfarin

The oral anticoagulant effect of warfarin may be increased which might lead to increase in prothrombin time and an increased risk of bleeding. Monitoring coagulation tests and adjustment of dose is needed.

- Carbamazepine:

Carbamazepine increases tramadol metabolism and because of the seizure risk associated with tramadol, co-administration is not recommended.

- Digoxin

Rare reports of digoxin toxicity, i.e. nausea, vomiting and cardiac arrhythmias, had been reported in post-marketing surveillance.

1.1.4 Anatomy and physiology of the rectum

The rectum can be considered as a hollow organ with a relatively flat wall surface, without villi and with only three major folds, the rectal valves. The rectal wall is formed by an epithelium which is one cell layer thick, and is composed of cylindrical cells and goblet cells which secrete mucus. The total volume of mucus is approximately 3 mL, spread over a total surface area of approximately 300 cm^2. The pH of the mucous is reported as approximately 7.2 (7-8).

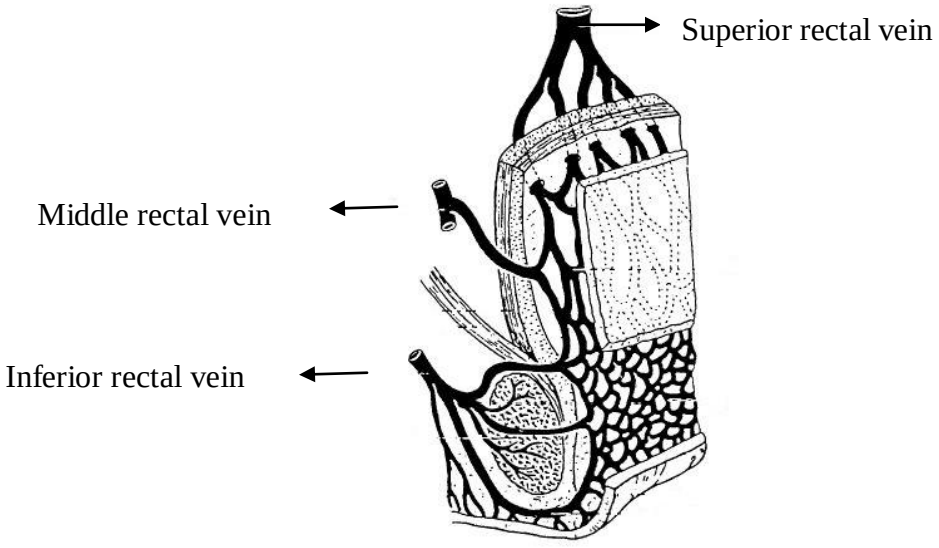

Figure 2 Vasculature of rectum (Lakshmi *et al.*, 2012)

There are three separate veins, which take part in the venous drainage from rectum. The lower and middle veins drain directly into the general circulation where as upper one drains into the portal vein, which flows to the liver (Tukker, 2002). Explaining more, the middle rectal vein drains from the lower part of the rectum whereas the inferior rectal veins drain from the anal canal rather than the rectum (Rosse and Gaddum-Rosse, 1997).

The positioning of a suppository in the rectum is critical in terms of exposure of drug to liver enzymes following absorption and subsequent metabolism. When the drug is absorbed in the lower parts of the rectum, it may enter the inferior and middle rectal veins, finally passing into the inferior vena cava, thereby bypassing the portal system and the liver. A drug absorbed from the upper parts of the rectum will probably be transported via superior rectal veins into the portal system and will pass through the liver before entering the systemic circulation. This fraction is thereby subjected to first-pass elimination in the liver (Boer and Breimer, 1979).

The mechanism of rectal absorption of drugs is not significantly different from those in the upper part of the gastrointestinal tract. The process of absorption is mainly passive diffusion whereas active transport processes, as seen in upper regions of GI tract, is not common in the rectal area (Tukker, 2002). Depending on chemical structure, drugs may cross the rectal wall either by absorption across the epithelial cell (transcellular) or via the tight junctions interconnecting the mucosal cell (paracellular) (Bergogne-Berezin and Bryskier, 1999).

The absorption from rectum is mainly dependent upon the molecular weight, lipid solubility and degree of ionization of molecules. Consequently, more absorption from rectal fluids of basic drugs was expected, since it would be largely un-ionized at rectal pH which is approximately 7.2 (Allen *et al.*, 2011, Lakshmi *et al.*, 2012).

1.2 Literature review and Rationale

Rectal route has long been accepted for opioid delivery (Zwaveling *et al.*, 2004), and considerable data of rectal opioids have been addressed in cancer and non-cancer patients (De Conno *et al.*, 1995, Mercadante *et al.*, 2005). Morphine immediate release rectal suppository had a faster onset and longer duration of action than those of oral dosage form (De Conno *et al.*, 1995). In addition, comparable AUCs obtained from morphine sustained release rectal suppositories and oral tablets has been revealed (Wilkinson *et al.*, 1992). Similarly, methadone rectal suppository was shown to be as effective as oral or intravenous administration in healthy volunteers (Dale *et al.*, 2004). Non-opioid agents, e.g. diclofenac and acetaminophen, were also studied for rectal suppositories. It had been reported that pre-operative rectal diclofenac provided effective analgesia and reduced opioid dose in post-operative period (Adarsh *et al.*, 2012). Reduced pain score was observed from naproxen rectal suppositories with a reduced dose of intravenous morphine (Kayacan *et al.*, 2004). In children, similar antipyresis had been reported from oral and rectal acetaminophen (Nabulsi *et al.*, 2005). It had also been reported that there was no difference in pain scores between tramadol suppositories 100 mg and paracetamol/codeine suppositories 1000/20 mg (Pluim *et al.*, 1999). Recent studies have shown comparable analgesic activity and tolerability of tramadol in oral and rectal dosage forms, thus rectal administration was suggested as an alternative route of administration for tramadol (Lintz *et al.*, 1998, Mercadante *et al.*, 2005).

Pharmaceutical formulation can play a major role in absorption and pharmacokinetics of rectally delivered drugs, especially suppository. Suppository base governs release of active constituent from the dosage forms and therefore has an impact on the availability of drug for absorption. Polyehtylene glycol (PEG, 400, PEG 4000, PEG 6000) (Tarimci and Ermis, 1998, Guneri *et al.*, 2004, Ozguney *et al.*, 2007, Saleem *et al.*, 2008, Reanmongkol *et al.*, 2011), Witepsol H15 (Tarimci and Ermis, 1998, Guneri *et al.*, 2004, Reanmongkol *et al.*, 2011) , Cocoa butter (Saleem *et al.*, 2008) were used as rectal suppository base for tramadol. Combined PEGs of different molecular weight

would provide the suppository base that can withstand the elevated temperature (Wade and Weller, 1994). Different ratios of PEG such as PEG 400: PEG 4000 (1:1) and PEG 400: PEG 6000, had been studied for tramadol suppository. Moreover, Witepsol H 15 was also used as a suppository base for tramadol (Saleem *et al.*, 2008, Reanmongkol *et al.*, 2011). Tramadol was rapidly released from both suppository bases, i.e. PEG and Witepsol H15, with more pronounced analgesic activity in PEG than Witepsol base was observed in rats (Reanmongkol *et al.*, 2011).

Rapid high plasma concentration after IV or oral administration can be associated with nausea and vomiting, which can be a major cause for discontinuation of tramadol use (Petrone *et al.*, 1999). Oral route had been accepted as the most convenient and simple route of administration. However, it is less applicable in the following situations: nausea and/or vomiting, dysphagia, severe constipation, and bowel obstruction (Mercadante *et al.*, 2005). Nausea and vomiting from tramadol is mediated by central effect (i.e. chemoreceptor trigger zone stimulation by serotonin) rather than direct effect on GI. Slower dose titration improved tolerability in patients who previously discontinued therapy due to nausea and/or vomiting (Petrone *et al.*, 1999). Beside this, parenteral administration has several disadvantages, including the requirement of more expertise and high cost. Rectal administration of tramadol can serve as an alternative of oral dosage form to avoid the adverse effect. Tramadol administered by either route provided adequate analgesia, producing similar clinical response in cancer patients (Mercadante *et al.*, 2005). No differences in adverse effects found between oral and rectal routes of tramadol, assured rectal route as a reliable, non-invasive method for patients unable to receive oral tramadol (Mercadante *et al.*, 2005). Few studies had reported clinical efficacy and pharmacokinetic of tramadol rectal suppository. Lintz *et al.* had shown that absorption of tramadol rectal suppositories was rapid and complete with comparable bioavailability to oral formulation (77% versus 67%) (Lintz *et al.*, 1998). Low variability in absorption and clearance of rectal tramadol had been revealed in children, in contrast to poor and more variable absorption of rectal morphine (Zwaveling *et al.*, 2004). The fact that tramadol metabolism to form an active metabolite ODT by

CYP 2D6 contributed its therapeutic response. Genetic or racial variability, mainly from CYP 2D6 mediated metabolism, could play a significant role in clinical effects. Pharmacokinetic of tramadol rectal administration has been addressed in a few studies (Lintz *et al.*, 1998, Zwaveling *et al.*, 2004). However, they are from different population, or pharmacologically active metabolite data are scarce (Lintz *et al.*, 1998).

Quantitative analysis of tramadol and its metabolites in biological samples had been accomplished using a variety of analytical approaches, such as gas chromatography (GC) with a nitrogen-selective detector, GC with a flame ionization detector and GC-mass spectrometry (MS) (Nobilis *et al.*, 2002). With regards to the analytical techniques for the determination from human plasma, HPLC methods with UV detection (Gan *et al.*, 2002), fluorescence (Gu and Fawcett, 2005, Rouini *et al.*, 2006, Ardakani and Rouini, 2007) or MS detection (Patel *et al.*, 2009), were reported. UV detection might be unsuitable for determination of low plasma concentration range commonly encountered in pharmacokinetic study. Mass spectrometry and fluorescence are common detectors used for quantification of low plasma concentration ranges of tramadol and related metabolites (Campanero *et al.*, 2004). Nobilis *et al*. reported that the fluorescence response was more than two orders of magnitude stronger than the response of the UV detector, when the appropriate wavelengths are chosen for excitation and emission (Nobilis *et al.*, 1996). Beside detector performance, a sample handling step is also necessary prior to the instrumental analysis of drugs in the bio-matrices in order to remove interfering compounds and to increase the selectivity and sensitivity of the analytical method (Nobilis *et al.*, 2002). For tramadol, liquid-liquid extraction (LLE) (Nobilis *et al.*, 1996, Gan *et al.*, 2002, Gu and Fawcett, 2005) or solid-phase extraction (SPE) (Gan and Ismail, 2001) were reported with excellent recovery. Common solvents used for LLE are diethyl ether, ethyl acetate, hexane, methylene chloride, chloroform, dichloromethane. One step LLE method had been employed with ethyl acetate for the determination of tramadol, *O*-desmethyltramadol (ODT), *N*-desmethytramadol (NDT) and *O*,*N*-didesmethyltramadol (ONDT) from human plasma, saliva and urine by HPLC method (Ardakani and Rouini, 2007). Several HPLC methods for the determination of

tramadol and its metabolites from biological fluids have been reported in the literature, with UV detection in plasma (Gan *et al.*, 2002) and in human breast milk (Kmetec and Roskar, 2003) or with fluorescence detection in plasma (Nobilis *et al.*, 1996, Gu and Fawcett, 2005, Rouini *et al.*, 2006) as well as in urine and saliva (Ardakani and Rouini, 2007).

Currently, tramadol is available in both oral and parenteral dosage forms in Thailand. Manufacturing and clinical uses of tramadol rectal suppository have not been reported. To examine pharmacokinetic of tramadol after rectal administration, rectal suppository of tramadol was formulated and evaluated in several aspects prior to pharmacokinetic study.

Objectives of the study

1. To explore pharmacokinetic properties of the tramadol rectal suppositories in healthy volunteers.

2. To formulate and evaluate tramadol suppository targeted for rectal delivery.

Chapter 2

Methods and Materials

2.1 Methods

2.1.1 Preparation of suppository

Bases used in this study were PEG and Witepsol H15 base. Combined PEG 400 and PEG 4000 bases were employed to yield appropriately melted base. In this study, PEG 400 (m.p. 4-8 ºC) and PEG 4000 (m.p. 53-56 ºC) were mixed in the ratio of 1:1 to accommodate body temperature since it was reported that melting point of the mixture was 43-49 ºC (Kaewnopparat and Kaewnopparat, 2009).

Suppositories containing 100 mg tramadol (approximately 113 mg of tramadol HCl) were prepared by the fusion method. Drug displacement values were first determined and the amount of suppository base required was calculated (Allen *et al.*, 2011). Then, base and tramadol HCl were accurately weighed according to the calculated weight for preparation of the predetermined amount of suppositories.

To prepare suppository with PEG base, equal amount of PEG 400 and PEG 4000 were melted in a 100 mL beaker over hot water bath. Tramadol HCl, equivalent to 100 mg tramadol was added to the melted base, sufficient to fill one cavity, and the content was stirred until the homogenous mixture was obtained. Then, the content was poured into mould and allowed to cool for 30-60 min at 25ºC. When solidified, suppository was removed, weighed and the displacement value of base in one suppository was calculated. Tramadol and suppository base for desired number of suppositories were accurately weighed and same process was repeated. Finally, formed suppositories were wrapped in aluminum foil and stored at 8-10º C. Similar process was performed for Witepsol H15 base suppositories.

Sustained release (SR) suppository was prepared in base containing Eudragit L100, a non-toxic and non-irritant and widely used film coating materials in oral formulations (Ahuja and Dong, 2005). Eudragit L 100 was added into either PEG or Witepsol H15 base to yield final concentrations of 1.5%, 2.5%, 3.5%, 5% w/w for PEG base, and 2.5%, 5%, 7.5%, 10% w/w for Witepsol H15 base. Drug displacement values of suppositories base with Eudragit L100 was calculated. Calculated weight of tramadol and base with Eudragit L 100 were added and these mixtures were heated to 70-80ºC in a water bath with occasional stirring until homogenous mixture was obtained. Then, it was poured in metal mould and allowed to cool at room temperature. Finally suppositories were removed, wrapped and stored as mentioned for conventional suppositories.

2.1.2 Evaluation of suppository

2.1.2.1 Disintegration test

USP tablet disintegration test apparatus was used to examine disintegration time of suppositories. Six suppositories were randomly chosen from each formulation and placed in the disintegration apparatus (Hanson Research, Chatsworth, USA, model no. 39-400-311). Distilled water maintained at 37 ± 1ºC was used as a disintegration medium. The time for disintegration was recorded when the suppository completely melted (Witepsol H15 base) or dissolved (PEG) in the medium.

2.1.2.2 Content Uniformity

Determination of drug content was performed according to previously reported method with slight modification (Saleem *et al.*, 2008, Ghorab *et al.*, 2011). The suppositories were melted using water bath heating in presence of distilled water. After final volume adjusted to 100 ml, the flask was continuously shaken for 30 minutes. Then the content was filtered prior to UV absorption at 272 nm measurement. Concentration was calculated from calibration curve prepared at 20-125 µg/mL. Content uniformity was

expressed as percentage of the actual concentration (mean ± SD of 3 determinations) (Guneri *et al.*, 2004).

2.1.2.3 Dissolution test

The release of tramadol from suppositories was studied using rotating basket dissolution apparatus (USP dissolution apparatus I). Dissolution apparatus consists of rotating basket dissolution apparatus (VK 7000, Vankel Technology group, North Carolina, USA) and UV instrument (Spectronic Genesys 5, Milton Roy, Rochester, USA). The release was performed at 37 ± 0.5ºC at 50 rpm in distilled water (900 mL) which served as dissolution medium. Five mililiter aliquot of the medium was withdrawn at 10 and 30 min interval for 2 and 6 hours for immediate and sustained release suppositories respectively. The sample was filtered and the amount of tramadol was determined by measuring ultraviolet absorption at 272 nm, using appropriate blank solutions. The concentration of tramadol was calculated from the standard curve, prepared at 20-125 µg/mL. Percent release of the suppository was reported as mean ± SD of six determinations. Additionally, varying pH of the dissolution medium was studied, i.e. phosphate buffer pH 7.2, 7.5 and 8. The study was performed by the same aforementioned procedures.

2.1.3 **Bioanalytical method development**

A number of HPLC methods with UV (Gan *et al.*, 2002), fluorescence (Gu and Fawcett, 2005, Rouini *et al.*, 2006, Ardakani and Rouini, 2007) and MS detection (Patel *et al.*, 2009) were employed for pharmacokinetic and bioequivalence study of tramadol and its metabolites in human plasma. The analytical method for the determination of tramadol, ODT, NDT in plasma used in this study was modified from that reported by Ardakani and Rouini *et al.*, 2007. Method modifications included sample preparation and chromatographic condition.

i) Chromatographic conditions

The HPLC system (CTO-10AS VP, Shimadzu, Tokyo, Japan) consisted of two LC-20AD VP pumps, a RF-10AXL fluorescence detector, an SIL-10ADVP autosampler, an SCL-10AVP oven controller, and a DGE-14A degassing unit. Separation was performed on a C18 column (Phenomenex Luna 150 mm x 4.6 mm i.d, 5µm). The detector operated with an excitation wavelength of 202nm and an emission wavelength of 310 nm. The mobile phase consisted of 0.05 M phosphate buffer (potassium dihydrogen orthophosphate and di-potassium hydrogen phosphate with 0.1 M Sodium chloride, 1% triethylamine) adjusted to pH 7 and acetonitrile in proportion 80:20 (v/v).

ii) Sample preparation

The frozen samples were allowed to thaw at room temperature before processing. Plasma sample 0.5 mL was spiked with 50 µl methocarbamol (IS) (1.0 µg/mL) prior to alkalinization with 100µl of 1M NaOH. After vortex mixing for 15 seconds, 3 mL ethyl acetate was added. The tube was vortexed again for 45 seconds, followed by horizontal shaking for 10 minutes. Then, the upper organic layer was separated into clean glass tube and evaporated under gentle stream of nitrogen. The residue was finally reconstituted with 300 µl of mobile phase, centrifuged at 4000 rpm for 10 minutes, transferred to injection vial and finally 50 µl aliquot was injected into the HPLC system.

iii) Validation

Bioanalytical method validation was performed according to US FDA guideline for bioanalytical method validation (US FDA, 2001). The key bioanalytical performance characteristics that must be validated for each analyte of interest in matrix include selectivity, accuracy, precision, recovery, calibration/standard curve and stability.

Selectivity

Selectivity was determined by analysis of blank plasma and plasma spiked with tramadol, ODT, and NDT. The chromatograms from the determination were compared for interfering peaks at the relevant retention times.

Calibration/standard curve

The calibration curve for tramadol were obtained by plotting the ratio of the tramadol peak area to the IS peak area against the tramadol concentration in ng/mL. Similarly for metabolites ODT and NDT, the peak area ratio was plotted against their respective concentrations expressed in ng/mL. The calibration curves were constructed over the range of 5, 10, 20, 50, 100, 200 and 500 ng/mL for tramadol and 5, 10, 20, 50 and 100 ng/mL for ODT and NDT.

Limit of detection (LOD) and lower limit of quantification (LLOQ)

LOD and LLOQ for each analyte were determined. The analyte peak of the LLOQ should be identifiable, discrete and reproducible with a precision of 20% and accuracy of 80-120%. The LLOQ on the basis of LOD can be predicted by following equation:

$$LLOQ = LOD + (10*SD)$$

Where, SD is standard deviation.

So, the lowest concentration of tramadol and metabolites from concentration range in calibration curve were selected to determine the LOD. Similarly, on the basis of the equation mentioned above, LLOQ for tramadol and metabolites were also determined. The accuracy and precision were calculated.

Accuracy and Precision

To assess the accuracy and precision, three different levels of plasma QC samples for tramadol, i.e. 25 ng/mL (low), 100 ng/mL (medium), and 500 ng/mL (high) were prepared. Two different levels of ODT and NDT concentrations were also prepared, i.e. 15 and 100 ng/mL. The intraday accuracy and precision were determined by repeated analysis of the QC samples on the same day (n=3). The inter day accuracy and precision were determined by repeated analysis of the QC samples on 4 different days. The mean value should be within ±15% of the actual value for accuracy and precision (US FDA, 2001).

Recovery

Recovery was determined by comparing the peak area of the tramadol (10, 50, 100 and 500 ng/mL), ODT (10, 50 and 100 ng/mL) and NDT (10, 50, 100 ng/mL) in plasma after extraction to that of each analyte obtained in an un-extracted standard solutions.

Stability

The stability was evaluated at 2 concentrations of tramadol (50 and 200 ng/mL) of tramadol and a single predetermined concentration of ODT and NDT (80 ng/ml). The study was performed at 37ºC using water bath. Aliquot 0.5 mL plasma was withdrawn at time 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours. For each analyte, obtained concentrations at specified time point were compared with the value determined at initial time (t=0).

2.1.4 Pharmacokinetic study

i) Participants recruitment

Healthy male and female volunteers aged 18-35 years and body mass index (BMI) 18-25 kg/m^2 were recruited to the study. They were screened based on medical history, physical examination, and standard laboratory test results (complete blood count, fasting blood glucose, blood urea nitrogen, serum creatinine, SGOT, SGPT, total bilirubin, alkaline phosphatase, HIV test). They were excluded if they smoked, had a history of alcohol and/or substance abuse, had an allergic history to tramadol or opiates, had participated in any clinical studies or were using any medications within at least 4 weeks prior to this study. In addition, volunteers were asked to refrain from drinking alcohol for at least 1 week prior to and throughout the study. All participants were informed about the risks and benefits, and details of the study. Written informed consent was obtained from all volunteers before any study procedures were performed. The study was conducted in accordance with the principles of good clinical practice and was approved by Ethics committee of Faculty of Medicine, Prince of Songkla University, Thailand.

Sample size calculation:

Sample size for the pharmacokinetic study is calculated on the basis of following equation:

$$n = (Z_{\alpha/2.}\, \sigma/E)^2$$

where,

n	=	Sample Size
$Z_{\alpha/2}$	=	1.96
σ	=	Standard deviation
E	=	Error of mean, 10% of the mean

A pilot study carried out prior to pharmacokinetic study have yielded C_{max} tramadol of 296.94 ± 63.51 ng/mL (n=5). Given σ = 63.51, and E = 29.69; the above equation had revealed the number of participants needed:

$$n = (1.96 * 63.51/29.69)^2$$
$$= 17.57$$

Thus, volunteers should be included in the study was 18

ii) Pharmacokinetic study

Ten males and 4 females (age 20.2 ± 0.9 years, body weight 59.9 ± 10.9 kg, BMI 20.75 ± 2.2 kg/m^2) were enrolled. After an overnight fast, a 100-mg tramadol suppository was administered to each volunteer by a nurse on the experiment day. The volunteers were asked to remain in the same position for 15 min after drug administration. Volunteers were also asked to hold defecation for 4 h thereafter. Venous blood samples (5 mL) were collected through a heparin locked indwelling catheter placed in a forearm vein and transferred to heparinized tubes at the following collection time points: pre-dose (time 0), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 hours after drug administration. All blood samples were immediately centrifuged at 4000 rpm for 10 min. The upper plasma samples was separated and transferred to a new tube. Plasma samples were stored at -20ºC until analysis.

Safety monitoring: Blood pressure, body temperature and pulse rate were recorded prior to drug administration, and every 2 hours after drug administration. Moreover, participants were interviewed for unusual symptoms periodically.

iii) Pharmacokinetic analysis

$AUC_{0-\infty}$, $T_{1/2}$, k_e, MRT, and Cl were estimated on the basis of non-compartmental approach by PK-Solver software. C_{max} and $T_{max,}$ were observed directly from plasma concentration time profiles.

a) AUC $_{0-\infty}$

$$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty}$$

where $AUC_{t-\infty} = C_{last}/k_e$

C_{last} = last time point concentration

k_e = the elimination rate constant

b) **Elimination rate constant (k_e) and half life ($T_{1/2}$)**

Elimination rate constant (k_e) and elimination half life ($T_{1/2}$) were obtained from the semilogarithmic plot of plasma concentration against time which is calculated by the following equation:

$$T_{1/2} = 0.693/ k_e$$

c) Mean Residence Time (MRT)

MRT represents the average time a drug molecule spends in the body before elimination.

For oral administration, $MRT = MAT + MRT_b$

$$= 1/k_a + 1/k_e$$

Where,

MAT: Mean absorption time

MRT_b: Mean residence time spent in body

k_a : Absorption rate constant

k_e : Elimination rate constant

2.1.5 Statistical analysis

Results were expressed as the mean ± SD. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Differences were considered statistically significant at $p < 0.05$.

2.2 Materials

The pure substances of tramadol hydrochloride was purchased from Sigma-aldrich Co. Switzerland, *O*-desmethyl tramadol and *N*-desmethyl tramadol were kindly supplied by Grunenthal GmbH (Germany). Methocarbamol were purchased from Sigma-Aldrich Co. China.

2.2.1 Chemicals and reagents

Tramadol Hydrochloride	Sigma Aldrich Co. Switzerland
O-desmethyltramadol	Grunenthal GmbH, Germany
N-desmethyltramadol	Grunenthal GmbH, Germany
Methocarbamol	Sigma-aldrich Co. China
Acetonitrile, HPLC grade	RCI Labscan Limited, Bangkok, Thailand
Methanol, HPLC grade	RCI Labscan Limited, Bangkok, Thailand
Ethyl acetate	Merck KGaA, Darmstadt, Germany
Dipotassium hydrogen phosphate	Emsure, Merck Co. Darmstadt, Germany
Potassium dihydrogen phosphate	Emsure, Merck Co. Darmstadt, Germany
Sodium Chloride	Emsure, Merck Co. Darmstadt, Germany

Triethylamine	Fluka Chemie GmbH, Switzerland
Nitrogen gas	Linde (Thailand) Public company limited
Polyethylene glycol (PEG) 400	S. Tong Chemicals Co. limited
Polyethylene glycol (PEG) 4000	S. Tong Chemicals Co. limited
Eudragit L 1000	Rohm Pharma, Germany

2.2.2 Instruments

HPLC system	Shimadzu HPLC system coupled with CTO-10ASVP, Shimadzu column oven SIL-20A, Prominence Autosampler RF-10AXL, Shimadzu fluorescence detector DGU-20AS, Prominence degasser
Analytical Column	Phenomenex Luna C18 column (4.6*150 mm, 5µm), USA
Guard Column	Phenomenex Security guard Cartridge, USA
Centrifuge	Hermle Labortechnik GmbH, Germany
Vortex	Scientific Industries Inc, USA
Micropipette	Gilson Inc, USA
Disintegration apparatus	Hanson Research, Chatsworth, USA
Dissolution apparatus	Vankel Technology group, North Carolina, USA)
UV instrument	Spectronic Genesys 5, Milton Roy, Rochester, USA

Chapter 3

RESULTS

3.1 In vitro evaluation of the suppositories

3.1.1 Disintegration test

Disintegration time for tramadol suppository in either PEG or Witepsol H15 bases for IR, and Eudragit L/PEG or Eudragit L/Witepsol H15 for SR characteristics were shown in Table 4. Disintegration time within 15 minutes in both PEG and Witepsol base indicated fast disintegration of the suppository. However, disintegration in Witepsol H15 base, either with or without Eudragit L, was faster than those formulated in PEG base. Results revealed the formulations tested conformed BP 2011 with the exception of a formulation with 5% Eudragit L100 in PEG base, which no disintegration was obtained (Table 4). This could be due to retardation of dissolution of hydrophilic PEG base by high concentration of lipophilic Eudragit L.

Table 4 Disintegration time (mean ± SD) for tramadol suppository using either PEG or Witepsol H15 as base for immediate release (IR), and with different concentrations of Eudragit L 100 for sustained release (SR) characteristics

PEG base					
Eudragit L 100 (%)	**0**	**1.5**	**2.5**	**3.5**	**5**
Disintegration time (min)	12.4 ± 1.6	11.5 ± 0.8	14.0 ± 1.9	29.8 ± 9.7	No disintegration
Witepsol H15					
Eudragit L 100 (%)	**0**	**2.5**	**5**	**7.5**	**10**
Disintegration time (min)	4.35 ± 0.1	4.02 ± 0.3	5.13 ± 0.1	5.31 ± 0.7	5.22 ± 0.5

3.1.2 Content Uniformity

As shown in Table 5, tramadol content in either IR or SR formulations were homogenous in all suppositories. It also complied with the requirement of BP with the test if not more than one individual content is outside the limits of 85 percent to 115 percent of the average content and none is outside the limits of 75 percent to 125 percent of the average content (British Pharmacopoea, 2011).

Table 5 Content uniformity (mean ± SD) of suppositories with Eudragit L100 in PEG and Witepsol H 15 base

PEG base					
Eudragit L 100 (%)	**0**	**1.5**	**2.5**	**3.5**	**5**
Content Uniformity (%)	108.5 ± 1.0	109.6 ± 3.1	100.6 ± 4.3	101.6 ± 2.6	103.9 ± 2.1
Witepsol H15					
Eudragit L 100 (%)	**0**	**2.5**	**5**	**7.5**	**10**
Content Uniformity (%)	93.4 ± 1.6	104.3 ± 4.3	107.7 ± 5.6	105.4 ± 7.2	100.0 ± 3.6

3.1.3 In vitro release study of suppository

Tramadol suppositories formulated in Witepsol H15 base showed rapid release of tramadol (88.62%) in 10 minutes compared to polyethylene glycol (PEG) base (68.74%). Both show complete release of tramadol (about 98%) in 20 minutes in distilled water medium as shown in Figure 3A and 3B. Thus, both Witepsol H15 and PEG yielded suppository with immediate release characteristics. Due to its inherent hydrophilicity, tramadol would be dissolved and partitioned in PEG base in a greater extent than in lipophilic Witepsol H15 base. Therefore, faster release rate was normally observed from Witepsol base compared to those from PEG base.

With Eudragit L100, rate of tramadol released from the preparation was delayed. The delayed release profiles depended on Eudragit concentration. For example,

approximately 90% of tramadol released from PEG base was observed at 3 and 5 hours with 3.5% and 5% Eudragit L100, respectively (Figure 3A). In case of Witepsol H15, the same extent of release was observed at 0.5 and 1.5 hours with 2.5% and 10 % Eudragit L100, respectively (Figure 3B). These results showed that delayed effect of tramadol release from Eudragit/Witepsol base was less pronounced than those from Eudragit/PEG base.

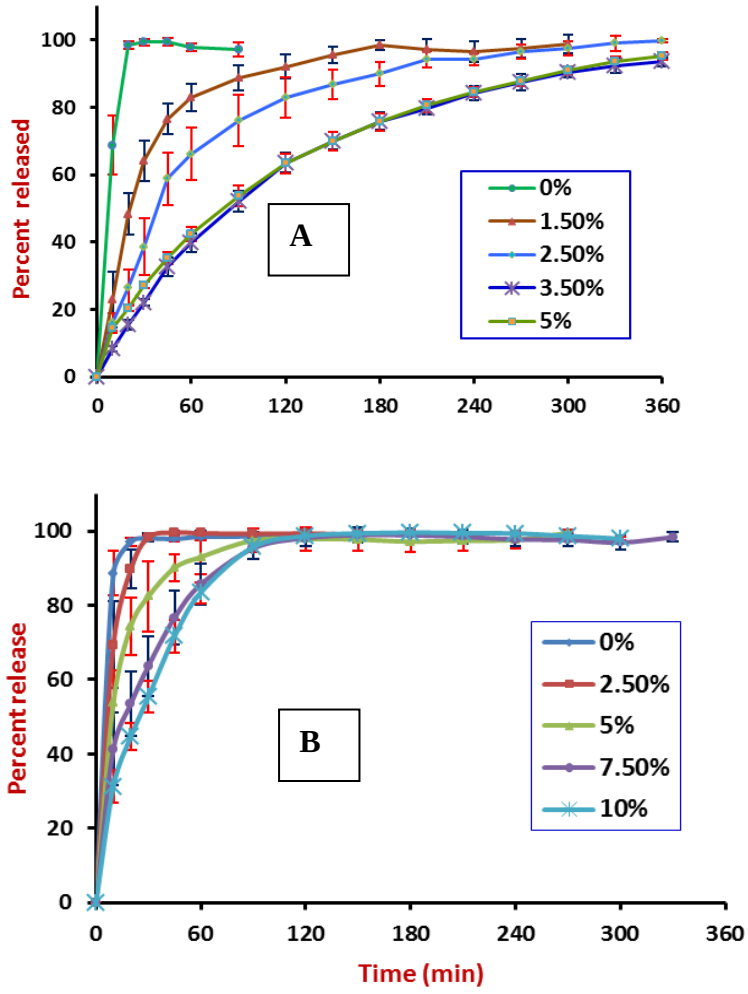

Figure 3 Release profiles of immediate as well as sustained release suppository in PEG (A) and Witepsol H15 (B) base in distilled water

Since pH of the rectum varies between pH 7-8, the conventional suppositories were also studied in dissolution medium of pH 7.2, 7.5, 8 and distilled water (pH 6.4), to check if pH of the dissolution medium affects the release of tramadol as shown in Figure 4. The percent release of tramadol is found to be significantly different (p <0.05) comparing between distilled water, pH 7.2, 7.5 and 8 in PEG and Witepsol H15 base suppository. This showed that the release of tramadol is affected by the pH of the dissolution medium. As the pH of the dissolution medium increased as shown in Figure 4A and 4B, the release of tramadol decreased.

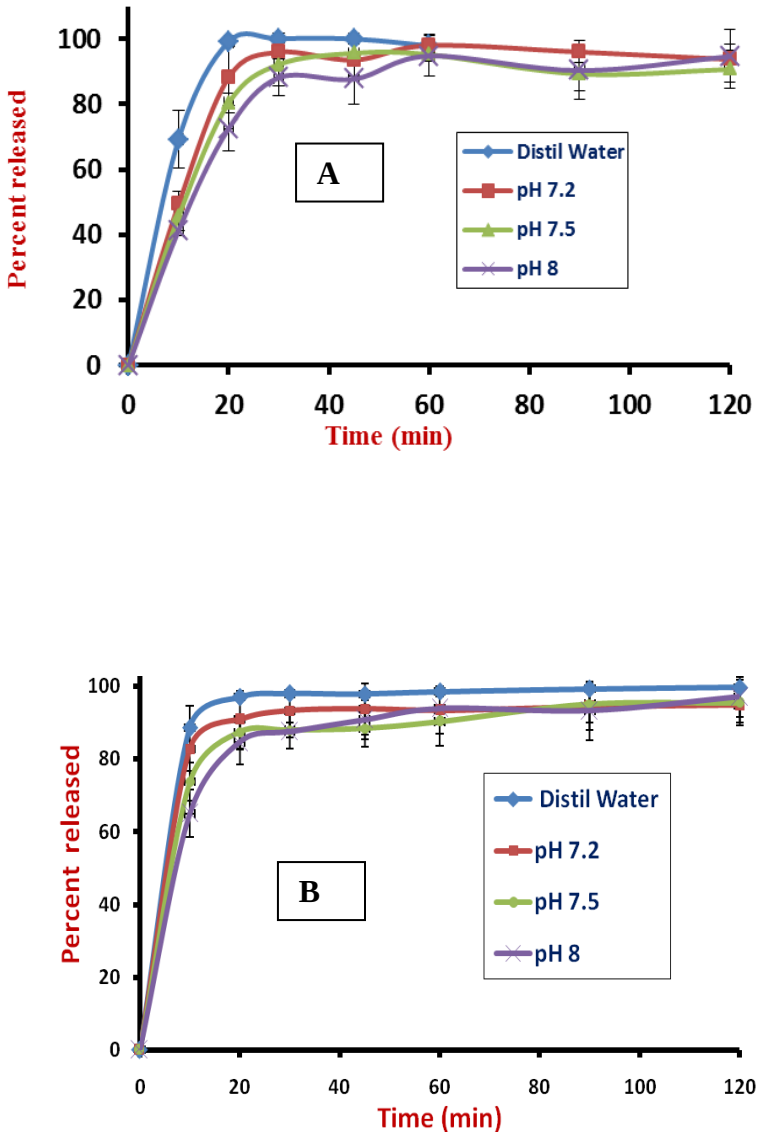

Figure 4 Release profiles of immediate release tramadol suppositories formulated in PEG (A) and Witepsol H15 base (B) in different dissolution medium (distilled water, phosphate buffer pH 7.2, 7.5 and 8)

3.2 Bioanalytical Method Validation

3.2.1 Selectivity/specificity

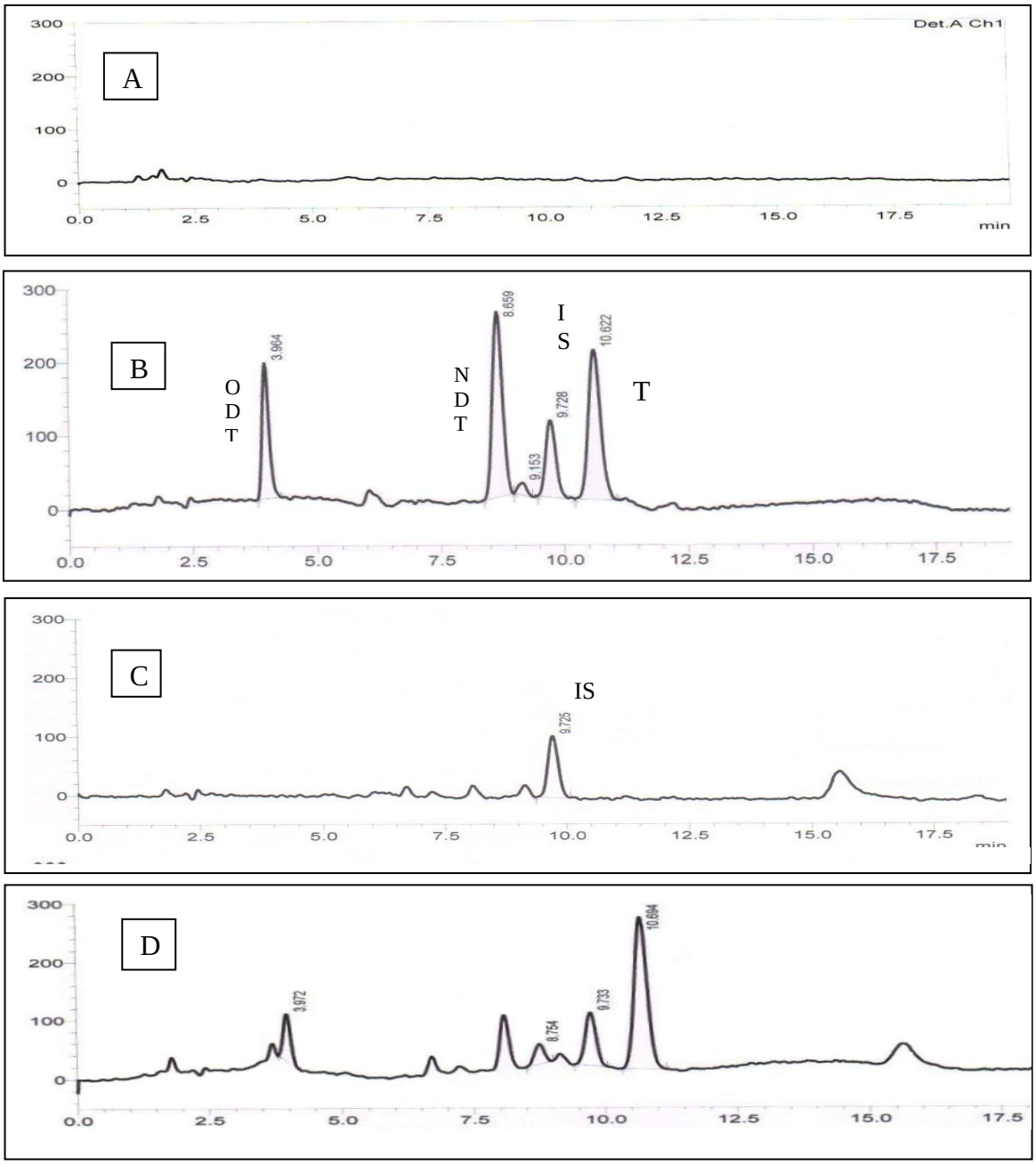

Figure 5 Chromatograms of blank human plasma (A); plasma spiked with tramadol, ODT, NDT, and IS each at 100 ng/mL (B); and plasma from healthy volunteer at t= 0 h (C) and 10 h after rectal administration of 100 mg tramadol suppository (D).

The separation achieved using liquid-liquid extraction method for tramadol and metabolites were presented in Figure 5. The absence of interfering endogenous peaks were observed at the retention times of all analytes in blank plasma. The retention times of ODT, NDT, IS and tramadol were 3.97, 8.75, 9.73 and 10.69 respectively. The absence of interfering endogenous components at the retention times of all analytes in blank plasma showed the selectivity sufficient for the pharmacokinetic study in human plasma.

3.2.2 Limit of detection and lower limit of quantification

Limit of detection and lower limit of quantification of tramadol and metabolites were determined.

Table 6 LOD and LLOQ of tramadol and metabolites

	Concentration tested (ng/mL)	Concentration found (ng/mL)	Accuracy (mean ± SD)	% CV
Tramadol				
LOD	5.19	5.01	96.4 ± 12.3	12.7
LLOQ*	12.5	14.0	112.9 ± 9.79	8.7
ODT				
LOD	2.7	3.03	112.4 ± 10.2	9.2
LLOQ**	5.4	5.48	101.4 ± 12.4	12.3
NDT				
LOD	5.23	4.87	93.2 ± 5.3	5.7
LLOQ***	8.4	7.71	91.8 ± 13.7	14

*predicted LLOQ for Tramadol, LLOQ = LOD +(10*SD)=11.38 ng/mL
**predicted LLOQ for ODT, LLOQ = LOD+(10*SD) = 5.2 ng/mL
***predicted LLOQ for NDT, LLOQ=LOD+(10*SD) = 7.79 ng/mL

3.2.3 Calibration Curve

Calibration curves prepared from plotting peak area ratios of tramadol (or ODT, NDT) and IS versus the respective plasma concentrations, were shown in Figure 6 by spiking plasma with tramadol, ODT and NDT. The calibration curves were linear as shown by r^2 value greater than 0.996.

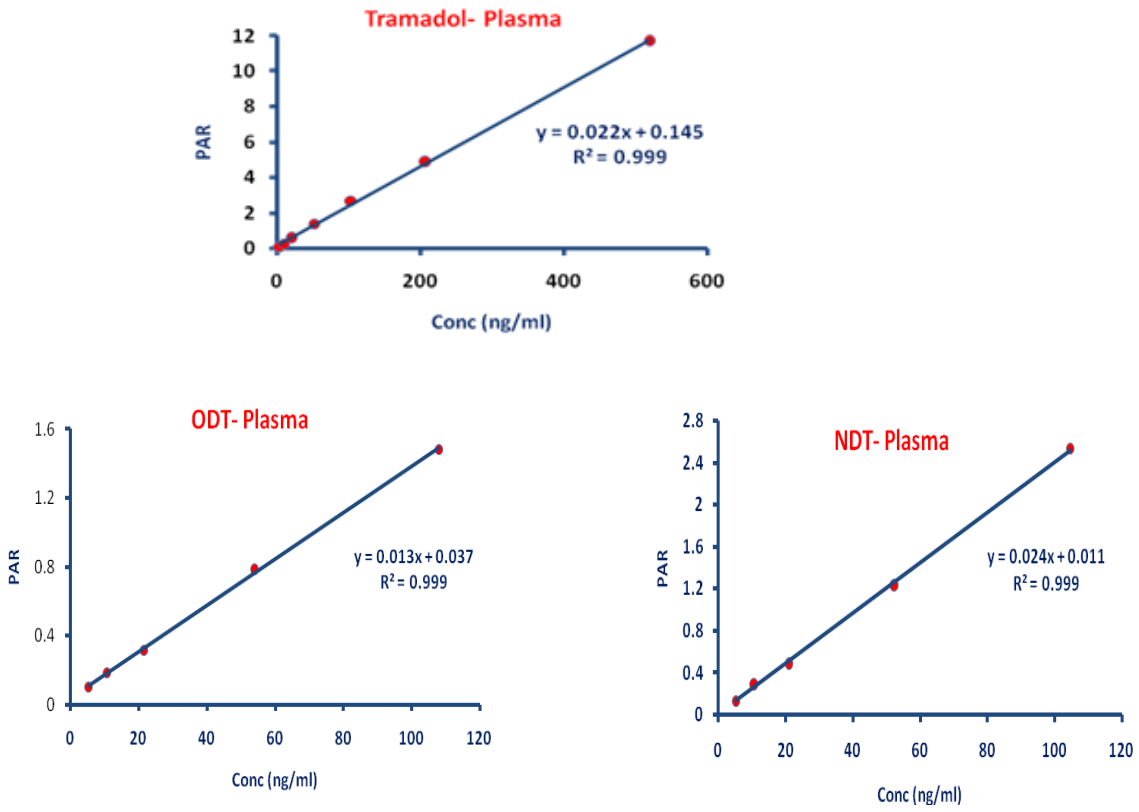

Figure 6 Calibration curves of tramadol, ODT and NDT

3.2.4 Accuracy and precision (Intra-day and Inter day)

Results for accuracy and precision were tabulated in Table 7, 8 and 9 for tramadol, ODT and NDT respectively. Since the criteria for acceptable precision and

accuracy were taken to be within ±15% (US FDA, 2001), these results were considered acceptable for this purpose of study.

Table 7 Intra- and Inter-day accuracy and precision for tramadol in human plasma

Conc[n]	Intra-day		Inter-day	
(ng/mL)	% Accuracy	%CV	% Accuracy	% CV
26	96.8	14.6	98.9	14.7
104	103.9	3.5	97.6	8.2
520	97.1	0.9	103.2	7.7

Table 8 Intra-day and inter-day accuracy and precision for *O*-desmethyl-tramadol (ODT) in human plasma

Conc[n]	Intra-day		Inter-day	
(ng/mL)	% Accuracy	% CV	% Accuracy	% CV
15.45	106.8	5.8	97.2	9.2
103	95.5	1.4	100.8	9.8

Table 9 Intra- and Inter-day accuracy and precision for *N*-desmethyl-tramadol (NDT) in human plasma

Conc[n]	Intra-day		Inter-day	
(ng/mL)	% Accuracy	% CV	% Accuracy	% CV
15.75	101.7	4.3	96.8	7.2
105	90.2	2.4	105.1	14.6

3.2.5 Recovery

As shown in Table 10, recoveries of tramadol and ODT were higher than NDT and methocarbamol. The recoveries of tramadol, ODT and NDT ranged between 79.7 to 85.7%, 73.5 to 78.6% and 65.1 to 89.1%, respectively. The mean recovery of methocarbamol was 60.3 % which was the lowest among the compounds extracted.

Table 10 Recovery of tramadol and metabolites from plasma

Tramadol				
Conc (ng/mL)	10.4	52	104	208
Recovery (%)	81.5 ± 12.5	85.7± 3.5	79.9 ± 3.9	79.7 ± 9.4
ODT				
Conc (ng/mL)	10.4	52	104	
Recovery (%)	73.5 ± 6.0	75.1 ± 5.8	78.6 ± 2.2	
NDT				
Conc (ng/mL)	10.6	53	106	
Recovery (%)	89.1 ± 21.5	70.5 ± 6.0	65.1 ± 1.6	
Methocarbamol				
Conc (ng/mL)	100			
Recovery (%)	60.3 ± 4.6			

3.2.6 Short term stability

Percent remaining of tramadol, ODT and NDT at different specified time points were plotted against time (Figure 7). No degradation was observed as the percent remaining of tramadol, ODT, and NDT at 12 hours were 102.1, 101.4 and 99.5% respectively.

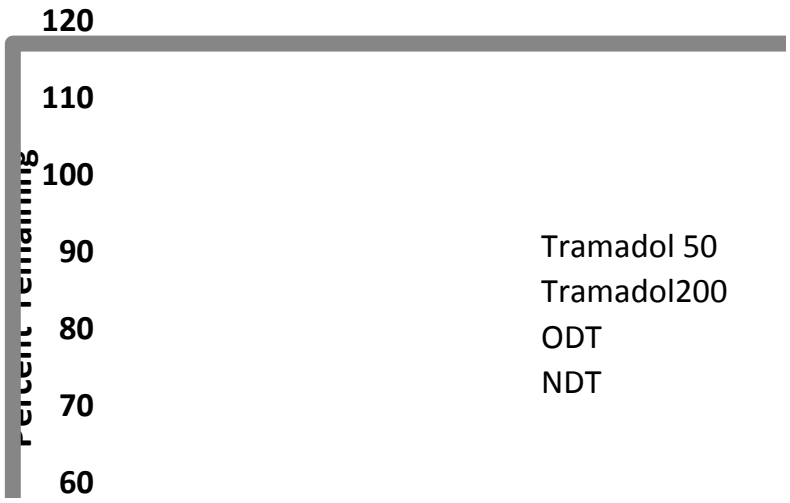

Figure 7 Stability profiles of tramadol, ODT, and NDT at 37ºC for 12 h in Plasma

3.3 Pharmacokinetic study in healthy volunteers

Fourteen volunteers (10 men and 4 women) participated in the study. Volunteers' characteristics were included in Table 11: age 20.2 ± 0.9 years, weight 59.9 ± 10.9 kg, BMI 20.75 ± 2.2 kg/m^2.

Table 11 Demographic data of study participants (n=14)

Number	Gender	Age (year)	Weight (kg)	Height (cm)	BMI (kg/m^2)
1	F	21.7	45.7	156	18.7
2	F	19.5	41.1	151	18
3	F	18.9	53	163	19.9
4	F	21	52	160	20.31
5	M	19.5	62.1	167	22.3
6	M	19.2	74.5	175	24.3
7	M	19.7	53.4	169	18.6
8	M	19.7	67.4	178	21.1
9	M	20	68.2	169	23.7
10	M	21.6	63.4	173	21.2
11	M	20.2	51.3	165	18.8
12	M	19.9	78	180	24.1
13	M	21.4	63.7	180	19.5
14	M	20.6	57.3	172	19.4

M : Male, F : Female, BMI : Body Mass Index

After rectal administration, C_{max} of 349.96 ± 80.4 ng/mL tramadol was attained in 3-5 h (T_{max} 3.93 ± 0.7 h) (Table 12). Plasma concentrations of ODT was observed as early as 0.5 h, with T_{max} ranged between 5-8 h (T_{max} 6.07 ± 1.5 h) (Table 12). NDT was detected within 1-2 hours in blood plasma with T_{max} slightly later than ODT (T_{max} 6.93 ± 1.7 h). Elimination half-lives of all compounds were similar (Table 12). Pharmacokinetic parameters were summarized in Table 12, of which plasma concentration-time profile was shown in Figure 8.

Table 12 Pharmacokinetic data (mean ± SD) obtained from healthy volunteers following administration of tramadol suppository (100 mg) (n=14)

Pharmacokinetic parameters	Tramadol	ODT	NDT
C_{max} (ng/mL)	349.96 ± 80.4	66.92 ± 30.2	22.98 ± 18.4
T_{max} (h)	3.93 ± 0.7	6.07 ± 1.5	6.93 ± 1.7
AUC $_{0\text{-}t}$ (µg.h/mL)	4.2 ± 1.2	0.92 ± 0.4	3.13 ± 0.3
$AUC_{0\text{-}\infty}$ (µg.h/mL)	4.69 ± 1.5	1.09 ± 0.4	0.40 ± 0.4
$T_{1/2}$ (h)	6.59 ± 1.4	8.3 ± 4.5	7.4 ± 2.9
k_e (h^{-1})	0.11 ± 0.02	0.10 ± 0.02	0.09 ± 0.03
MRT (h)	11.09 ± 1.96	12.95 ± 2.69	14.92 ± 4.27
Cl/F (L/h)	23.47 ± 7.71	104.7 ± 40.17	550.07 ± 516.99
V_d/F (L)	219.63 ± 72.41	1245.47 ± 849.34	4421.37 ± 2535.84

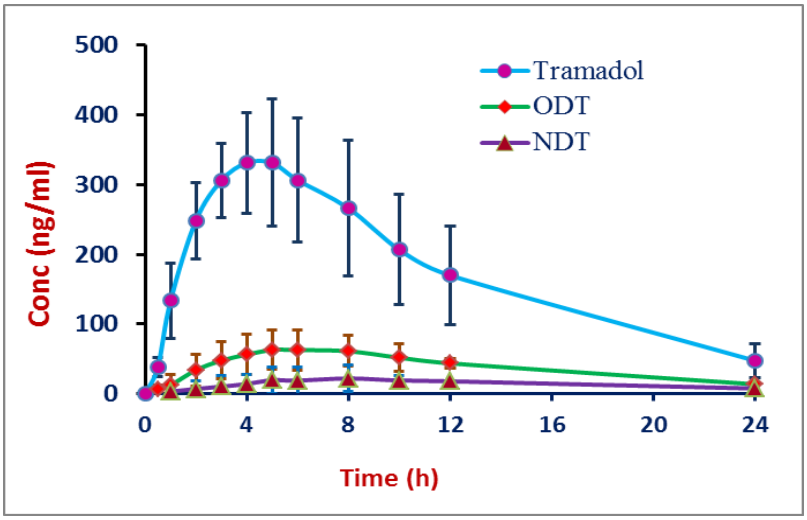

Figure 8 Concentration time profile of tramadol and its metabolites, ODT and NDT, from healthy volunteers after rectal administration of a 100 mg tramadol suppository (n=14)

Tolerability

Adverse effects observed were: dizziness (57.1%), drowsiness (35.7 %), nausea (35.7 %), and vomiting (28.6 %). The vomiting was commonly observed during peak plasma concentration time of tramadol, although a few cases reported at 10 h post administration. Seven volunteers (50%) experienced the defecation with some case of mild diarrhea. All events resolved in the same day without treatment, and did not lead to drop out.

Chapter 4

DISCUSSION

4.1 Formulation

4.1.1 In vitro release study

Due to lipophilic nature of Witepsol H15 which melted readily at body temperature, rapid release of drug could be obtained from Witepsol H15. While hydrophilic PEG needs to be dissolved in rectal fluid, the release of tramadol from PEG was slower than those from Witepsol (Reanmongkol *et al.*, 2011). However, fast release of tramadol within 20 min from conventional formula (both PEG and Witepsol) suggested IR characteristics according to FDA guideline for immediate oral solid dosage form. For IR criteria, not less than 85% of drug should be released within 60 minutes (US FDA, 1997).

Tramadol (pKa 9.2) would be less ionized as the pH of the dissolution medium increases, leading to decreased solubility of the drug. This could be the main reason for reduced drug release at increasing pH. At the same pH, release of tramadol in Witepsol based suppository was faster (64-82%) compared to PEG base (40-49%). This can be explained by the same reason for the disintegration data; rapid melting of Witepsol caused faster release than slowly dissolving PEG base in aqueous medium.

With Eudragit L100, rate of tramadol released from the preparation was delayed. The delayed release profiles depended on Eudragit concentration. The results showed that delayed effect of tramadol release from Eudragit/Witepsol base was less pronounced than those from Eudragit/PEG base. Since PEG is hydrophilic but Eudragit L100 is practically insoluble in water, in Eudragit/PEG base, a cage like surface resulting from slowly dissolving PEG was proposed (Tarimci and Ermis, 1998). Tramadol, which is readily soluble in water, can be trapped in this network structure and gradually dissolved. However, high affinity of lipophilic Witepsol and Eudragit offered the least chances of the cage like structure to form in case of combined Eudragit/ Witepsol base. In addition to in vitro release study, a recent study in rats has revealed that the latency of analgesic activity was prolonged in PEG

base compared to Witepsol H15 base (Reanmongkol *et al.*, 2011). Thus, formulation of conventional tramadol suppository in PEG base had been selected for pharmacokinetic study.

4.2 Bioanalytical Method development and validation

The analytical method for the determination of tramadol and metabolites in plasma was performed according to previously reported liquid-liquid extraction (Ardakani and Rouini, 2007), with some modification. Since tramadol would be mostly unionized at basic pH, thus alkaline condition would be essential for extraction of tramadol and its metabolites by organic solvents. Alkalinization of plasma sample to improve extraction efficiency was obtained from the addition of 1.0 M NaOH. A small separate study varying NaOH concentration had shown that the addition of 1.0 M NaOH gave the highest recovery of the extraction by ethyl acetate. Thus, the 1M NaOH was used for extraction process.

Chromatographic condition employed in this study yielded well-separated peaks of tramadol, ODT and NDT, with a total run time of 20 min. The absence of interfering peaks at retention time of analytes showed the selectivity of developed chromatographic method. The linearity of calibration curve over the concentration ranges studied was demonstrated by coefficient of correlation (r^2) >0.996 obtained for the regression line, shown by the equation for each compound, for tramadol y = 0.0223x + 0.0831 (r^2=0.9988); for ODT, y = 0.0118x + 0.0384 (r^2=0.9985) and for NDT, y = 0.0205x + 0.0243 (r^2 = 0.9988)

Lower limit of quantification of tramadol (14.0 ng/mL), ODT (5.48 ng/mL), NDT (7.71 ng/mL) were adequate for pharmacokinetic study. Fluorescence detection might be improved the sensitivity of this method compared to UV detection, which LLOQ 50 ng/mL was reported (Gan and Ismail, 2001). This method, however, showed a higher LLOQ than a previously reported method (LLOQ 2.5 ng/mL) (Ardakani and Rouini, 2007). This might be due to some limitation in analytical work, which might be related to the instrument, such as detector (light source) and analytical column.

The compromised internal standard recovery might be beneficial for the recovery of tramadol, which is the major analyte of interest, along with other metabolites. The precision and accuracy of tramadol, ODT and NDT were within acceptable limits (±15%) as specified by FDA guideline for bioanalytical method validation, these results were considered acceptable for the purpose of study. In terms of stability, no significant degradation of tramadol, ODT and NDT were observed, as shown by percent remaining of compounds after 12 hours. This indicated the stability of tramadol and its metabolites during 4-6 h of sample preparation.

4.3 Pharmacokinetic study

In this study, C_{max} of tramadol after rectal suppository administration of 350 ± 80.4 ng/mL corresponded to those reported by Lintz et al. (294 ±50 ng/mL), who performed the rectal tramadol delivery in Germans (Lintz et al., 1998). However, PK parameters of neither ODT nor NDT was reported in that study. Maximum plasma concentration observed in this study was similar to those reported by oral administration of the same dose (Ardakani and Rouini, 2007) (Table 13). It has been reported that C_{max} of tramadol following 100 mg oral administration is approximately 300 ng/mL (Grond and Sablotzki, 2004). Thus, C_{max} of tramadol after rectal administration was well agreed with those reported rectal and oral administration of the same dose. Time to maximum plasma concentration (T_{max}) of 3.93 ± 0.73 h, agreed well with those study performed by rectal tramadol (Lintz et al., 1998), but longer than those from IR oral administration (Table 13). T_{max} for oral administration was reported to be 1.6-1.9 hrs (Grond and Sablotzki, 2004). Delay in absorption could be slow release of tramadol from PEG base secondary to the gradual dissoving of PEG in reduced volume of rectal fluid. T_{max} obtained from rectal administration was comparable to those from SR oral administration, but with higher C_{max} (Table 13).

Table 13 Pharmacokinetic data (C_{max}, T_{max}, AUC and $T_{1/2}$) of tramadol after administration of tramadol 100mg in different dosage forms

Parameters	Experimental data	Lintz *et al.*, 1998	Ardakani and Rouini, 2007	Gu and Fawcett, 2005
Dosage	**Suppository**	Suppository	IR tablet	SR tablet
C_{max} (ng/mL)	**350 ± 80.4**	294 ± 50	314 ± 53.3	215.5
T_{max} (h)	**3.93 ± 0.7**	3.3 ± 1.3	1.8 ± 0.4	5.5
$AUC_{0-\infty}$ (µg.h/mL)	**4.69 ± 1.5**	2.9 ± 0.3	3.0 ± 0.7	2.6
$T_{1/2}$ (h)	**6.59 ± 1.4**	5.72 ± 1.1	7.1 ± 0.4	6.1

Mean C_{max} of ODT observed in the study was 66.9 ± 30.2 ng/mL, which is roughly similar to concentration found after oral administration (Rouini *et al.*, 2006, Ardakani and Rouini, 2007) (Table 14). The T_{max} of ODT took approximately 2 h later than those from tramadol. The maximum plasma concentration of ODT is about 19.12% and $AUC_{0-\infty}$ is 23.24% of the corresponding parameter of tramadol, which is almost similar to previously reported data for oral administration, where C_{max} of ODT is 24% and AUC is 32 % of respective parameters of tramadol (Ardakani and Rouini, 2007).

Compared to other reported parameters, C_{max} and T_{max} of ODT obtained in this study were quite similar to oral SR tablets, while $AUC_{0-\infty}$ and $T_{1/2}$ agree well to both oral SR and IR forms (Table 14). Thus, rate of absorption from rectal tramadol resembled sustained release characteristic of oral formulation. Since the analgesic property of tramadol was mainly ascribed to ODT, the absorption patterns from rectal administration should be beneficial in terms of consistent pain control and reduced frequency of administration.

Table 114 Pharmacokinetic data of ODT after administration of tramadol 100 mg in different dosage forms

Parameters	**Experimental data**	Ardakani and Rouini, 2007	Gu and Fawcett, 2005
Dosage form	Suppository	IR tablet	SR tablet
C_{max} (ng/mL)	**66.9 ± 30.2**	88.6 ± 23.7	58.9
T_{max} (h)	**6.07 ± 1.5**	2.4 ± 0.7	7
$AUC_{0-\infty}$ (µg.h/mL)	**1.09 ± 0.4**	1.1 ± 0.3	0.9
$T_{1/2}$ (h)	**8.28 ± 4.5**	7.4 ± 1.1	7.1

Mean C_{max} of NDT observed in this study (22.97 ± 18.4 ng/mL) was similar to the reported concentration for oral IR tablets (24-35 ng/mL) (Table 15). C_{max} and $AUC_{0-\infty}$ of NDT was accounted for 6.6% and 8.6%, respectively, of the corresponding parameter of the parent compound. While C_{max}, $AUC_{0-\infty}$, and $t_{1/2}$ of NDT obtained from this study were similar to those from IR tramadol tablet, T_{max} appeared to be greater than those of oral IR formulation (Table 15). NDT appeared to form at similar rate to ODT, since similar T_{max} of both metabolites were observed (Table 14 & 15). Comparable T_{max} of ODT and NDT was reported in oral IR tablets (Rouini *et al.*, 2006, Ardakani and Rouini, 2007).

Table 125 Pharmacokinetic data of NDT after administration of tramadol 100 mg in different dosage forms

Parameters	**Experimental data**	Ardakani and Rouini, 2007	Rouini *et al.*, 2006
Dosage form	Suppository	IR tablet	IR tablet
C_{max} (ng/mL)	**22.97 ± 18.3**	24.8 ± 15.1	35.5±4.0
T_{max} (h)	**6.93 ± 1.7**	2.8 ± 1.1	3.8± 0.6
$AUC_{0-\infty}$ (µg.h/mL)	**0.40 ± 0.4**	0.33 ± 0.2	0.4 ± 038
$T_{1/2}$ (h)	**7.38 ± 3.0**	10.3 ± 2	6.1 ± 1.2

Mean residence time (MRT) represents the average time a drug molecule spends in the body before elimination. For extravascular route, MRT is the sum of time spent in the administration site (mean absorption time) as well as time spent in rest of body (Jambhekar and Breen, 2009). In the study, the MRT of tramadol after rectal suppository was 11.09 ± 1.9 h, similar to those calculated for tramadol suppository (9.8h) reported by Lintz (Lintz *et al.*, 1998). MRT from subcutaneous tramadol administration was reported 7.77 ± 3.73 h (Dooney *et al.*, 2014). The shorter MRT from subcutaneous route than rectal route reflected faster absorption from administration site by subcutaneous injection.

Clearance (CL/F) of tramadol correlated to those reported values for oral administration (30.7 ± 3.6 L/h) (Ardakani and Rouini, 2007). Greater Cl/F of ODT and NDT compared to parent tramadol indicated dissimilar distribution kinetics of the compounds. According to our results, greater Vz/F of ODT and NDT compared to those of tramadol was speculated. However, Vz/F of tramadol rectally administration was comparable to those reported by Lintz (216 ± 23 L) (Lintz *et al.*, 1998).

Chapter 5

CONCLUSION

Two types of suppository bases were employed in preparing tramadol suppositories: PEG and Witepsol H15. Although the release from Witepsol H15 was generally greater than from PEG, almost 90 % release from both conventional formulations obtained in 30 min indicated IR characteristics. When Eudragit L100 was included in these bases, delayed release of tramadol was virtually obtained, which the most pronounce effect obtained from PEG/Eudragit L 100 formula. Rectal suppository containing 100 mg tramadol in PEG base was prepared for the pharmacokinetic study. Simultaneous determination of tramadol, ODT, and NDT concentrations in plasma was achieved using HPLC coupled with fluorescence detector (emission/ excitation wavelength 202 nm/310 nm). The analytes were extracted from plasma sample using ethyl acetate under alkali condition. With single extraction procedure, recoveries obtained were 79.7-85.7 %, 73.5-78.6%, 65.1-89.1 %, and 60.3% for tramadol, ODT, NDT, and IS, respectively. The compounds were separated using C18 (Phenomenex C18 Luna, 150X4.6 mm, 5µm), eluted by mobile phase consisting acetonitrile-0.05 M phosphate buffer, pH 7 (20:80) at 30^{o}C (1.0 ml/min). Methocarbamol was used as an internal standard. With an isocratic elution, ODT, NDT, methocarbamol (IS) and tramadol were eluted at 3.97, 8.75, 9.73 and 10.69, respectively with a run time of 20 min. LLOQ of tramadol (14.0 ng/mL), ODT (5.48 ng/mL), NDT (7.71 ng/mL); and accuracy and precision within ±15% for all compounds were obtained. Stability was shown by percent remaining of tramadol (102.1%), ODT (101.4%) and NDT (99.5%) after 12 hours at 37ºC revealing no degradation at experimental condition. The analytical method employed therefore was simple, fast, and adequate for pharmacokinetic study.

Fourteen healthy participants were enrolled in pharmacokinetic study. Following 100 mg tramadol rectal suppository administration, C_{max} of tramadol, ODT and NDT were 349.96 ± 80.4 ng/ml, 66.92 ± 30.2 ng/ml and 22.98 ± 18.4 ng/ml respectively. Similarly, T_{max} were 3.93 ± 0.7 h, 6.07 ± 1.5 h and 6.93 ± 1.7h. Mean

residence time (MRT), Vz/F and Cl/F for tramadol were 11.09 ± 1.96 h, 219.63 ± 72.41 L and 23.47 ± 7.71 L/h. Parameters, such as C_{max}, AUC, and $t_{1/2}$, of tramadol, ODT, and NDT from this study were similar to those values from previous rectal or oral IR tablets. Increase T_{max} observed in this study, however, resembled those from oral SR preparations. This suggested comparable extent of exposure of rectal and oral formulations, in spite of slower absorption rate of the rectal preparation. Although usefulness for acute pain management was not implied for rectal tramadol, its advantage in treating chronic pain was more likely.

Overall, this study revealed the pharmacokinetic parameters of tramadol and its metabolites (ODT and NDT). We believe the results from this study could provide useful information for clinicians in implementing tramadol suppositories in patients unsuitable for oral or parenteral administration. However, further study comparing the analgesic efficacy from rectal and oral dosage forms should be conducted to warrant clinical use of tramadol rectal suppository.

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APPENDIX

Table A1 Plasma concentration of tramadol after rectal administration of 100 mg tramadol suppository in healthy volunteers (N=14)

	Time after administration (h)											
Subject	0	0.5	1	2	3	4	5	6	8	10	12	24
1	0.0	16.4	135.2	182.0	317.2	401.5	479.4	466.0	450.7	409.5	320.0	104.7
2	0.0	45.4	131.2	287.8	401.9	465.3	445.3	410.4	319.2	247.2	221.2	34.4
3	0.0	50.2	118.6	289.6	299.1	389.5	414.2	337.3	297.6	257.4	249.6	67.1
4	0.0	42.1	118.5	245.7	313.2	336.8	420.0	405.0	397.3	236.8	220.4	39.6
5	0.0	42.9	176.4	255.0	274.3	262.6	219.2	216.3	162.6	143.8	103.5	34.7
6	0.0	48.3	151.8	171.5	205.8	212.0	201.6	175.4	144.9	115.9	81.8	25.0
7	0.0	39.3	109.6	200.1	269.5	325.1	289.3	270.6	205.7	147.0	105.0	24.2
8	0.0	38.5	76.5	190.9	267.3	303.3	287.8	254.5	229.8	142.7	120.1	27.4
9	0.0	10.1	86.8	210.0	282.3	268.0	249.4	224.2	151.3	125.1	86.4	21.3
10	0.0	34.1	97.5	250.6	257.0	241.4	224.7	198.4	169.4	147.3	121.8	38.7
11	0.0	27.4	152.3	338.7	353.3	390.5	380.5	372.8	371.8	273.4	194.0	46.7
12	0.0	32.8	107.5	225.3	321.0	289.1	285.3	286.0	242.8	219.3	152.6	81.8
13	0.0	43.0	293.8	309.7	335.5	366.2	359.1	323.4	258.7	196.3	187.6	63.1
14	0.0	62.0	111.3	322.2	386.5	389.1	381.9	353.2	326.7	232.1	215.4	55.5
Mean	0.0	38.0	133.4	248.5	306.0	331.4	331.3	306.7	266.3	206.7	170.0	47.5
SD	0	13.5	53.5	54.5	52.7	72.3	91.4	88.5	97.4	79.2	71.0	24.4
%CV	0.0	35.4	40.1	21.9	17.2	21.8	27.6	28.9	36.6	38.3	41.8	51.5

Table A2 Plasma concentration of ODT after rectal administration of 100 mg tramadol suppository in healthy volunteers (N=14)

	Time after administration (h)											
Subject	0	0.5	1	2	3	4	5	6	8	10	12	24
1	-	-	17.0	24.5	46.1	57.2	74.7	81.5	86.6	83.1	75.5	31.7
2	-	-	13.2	50.3	79.1	97.8	109.9	104.6	99.6	88.3	67.5	16.2
3	-	-	9.5	30.8	41.7	52.8	58.9	51.5	51.7	47.3	45.3	14.2
4	-	6.0	17.5	47.9	58.8	71.1	93.6	100.8	110.4	72.9	71.3	17.6
5	-	5.9	22.4	40.6	40.2	45.6	42.8	46.9	39.0	35.4	27.6	11.1
6	-	-	9.0	23.6	31.7	37.4	43.8	42.4	37.3	34.0	27.4	8.0
7	-	-	19.9	51.4	70.5	86.8	89.8	89.1	74.1	57.7	36.4	7.2
8	-	-	13.0	35.1	51.4	62.2	62.3	60.2	58.9	41.5	30.1	8.5
9	-	-	16.3	55.0	93.4	101.1	99.8	91.7	76.7	65.0	50.9	12.6
10	-	-	4.8	19.1	23.9	26.7	29.3	28.1	26.9	27.0	24.3	10.1
11	-	-	14.5	51.1	58.8	68.4	77.1	81.1	86.4	74.9	66.8	20.3
12	-	-	3.6	7.5	16.6	19.3	22.9	23.1	26.6	24.8	17.4	15.7
13	-	-	6.6	14.3	21.7	21.9	28.0	27.4	24.8	24.1	24.9	7.5
14	-	-	5.8	24.6	34.7	40.0	48.6	50.3	56.8	50.0	47.9	11.1
Mean	0.0	6.0	12.4	34.0	47.8	56.3	63.0	62.8	61.1	51.9	43.8	13.7
SD	0	0.1	5.9	15.6	22.5	26.7	28.4	28.3	28.4	22.0	19.9	6.6
%CV	0.0	1.4	47.9	45.8	47.1	47.3	45.2	45.1	46.4	42.5	45.3	48.0

"-"represents peak of ODT was not observed during those time intervals.

Table A3 Plasma NDT concentration after rectal administration of 100 mg tramadol suppository in healthy volunteers

	Time after administration (h)											
Subject	0	0.5	1	2	3	4	5	6	8	10	12	24
1	-	-	-	6.9	17.3	22.6	56.7	44.7	55.9	53.3	53.7	23.5
2	-	-	-	5.8	9.0	10.6	13.2	12.1	10.2	10.5	7.9	-
3	-	-	5.8	26.9	23.2	48.3	53.5	52.8	67.8	68.1	60.6	12.6
4	-	-	1.7	6.1	7.8	12.7	15.3	16.1	20.7	9.0	16.8	3.3
5	-	-	2.8	6.6	7.1	7.5	8.4	9.0	7.5	6.0	4.6	0.7
6	-	-	-	6.6	11.9	16.2	15.8	17.8	16.8	13.9	12.6	4.8
7	-	-	4.0	10.4	13.7	16.6	19.3	20.0	18.2	15.4	10.0	3.1
8	-	-	-	3.3	6.4	7.9	9.3	8.2	9.2	4.7	5.9	-
9	-	-	-	3.1	4.9	7.8	6.0	8.9	6.3	5.1	4.0	-
10	-	-	3.1	12.2	18.4	20.4	29.6	27.1	33.0	33.4	31.1	16.3
11	-	-	2.0	6.5	9.1	10.9	16.5	16.2	16.9	14.5	11.8	3.0
12	-	-	-	4.0	11.0	11.9	15.5	17.7	27.3	25.3	23.0	7.5
13	-	-	-	0.1	2.2	3.4	9.5	11.2	10.0	6.3	7.0	1.4
14	-	-	-	0.1	3.4	1.9	5.1	4.9	9.2	3.1	2.0	-
Mean	-	-	3.2	7.0	10.4	14.2	19.5	19.0	22.1	19.2	17.9	7.6
SD	-	-	1.5	6.6	6.0	11.5	16.3	13.9	18.7	19.7	18.4	7.5
%CV	-	-	45.7	94.1	58.1	80.8	83.4	73.1	84.5	102.7	102.9	98.6

"-" represents peak of NDT was not observed during those time intervals.

Table A4 Pharmacokinetic parameters of tramadol after rectal administration of 100 mg tramadol suppository in healthy volunteers

Subject	C_{max} (ng/mL)	T_{max} (h)	AUC $_{0-\infty}$ (µg.h/mL)	k_a (h^{-1})	k_e (h^{-1})	$T_{1/2}$ (h)	MRT (h)	Cl/F (L/h)	Vz/F (L)
1	479.38	5.00	7.87	0.51	0.10	7.23	13.29	12.71	132.53
2	465.28	4.00	5.47	0.51	0.14	5.00	9.32	18.27	131.78
3	414.17	5.00	5.99	0.67	0.09	7.51	12.35	16.67	180.55
4	420.04	5.00	5.37	0.39	0.14	5.00	9.85	18.63	134.29
5	274.26	3.00	3.38	0.9	0.10	6.93	10.80	29.57	295.46
6	211.97	4.00	2.66	0.75	0.11	6.34	10.21	37.53	343.36
7	325.09	4.00	3.37	0.59	0.13	5.48	9.24	29.68	234.67
8	303.27	4.00	3.49	0.55	0.12	5.79	9.80	28.68	239.61
9	282.29	3.00	2.87	0.56	0.12	5.64	9.22	34.78	282.75
10	256.99	3.00	3.48	0.99	0.10	7.25	11.66	28.69	300.35
11	390.52	4.00	5.45	0.6	0.12	5.61	10.12	18.33	148.25
12	321.02	3.00	5.36	0.53	0.07	10.39	16.33	18.67	279.72
13	366.16	4.00	5.4	0.59	0.09	7.73	12.16	18.51	206.51
14	389.05	4.00	5.59	0.75	0.11	6.4	11.00	17.87	165.01
Mean	349.96	3.93	4.69	0.63	0.11	6.59	11.09	23.47	219.63
SD	80.45	0.73	1.49	0.16	0.02	1.43	1.96	7.71	72.41
% CV	22.99	18.58	31.79	25.69	18.79	21.63	17.71	32.87	32.97

Table A5 Pharmacokinetic parameters of ODT after rectal administration of 100 mg tramadol suppository in healthy volunteers

Subject	C_{max} (ng/mL)	T_{max} (h)	AUC $_{0-\infty}$ (µg.h/mL)	k_e (h^{-1})	$T_{1/2}$ (h)	MRT (h)	Cl/F (L/h)	Vz/F (L)
1	86.57	8.00	1.85	0.07	9.9	17.75	54.1	772.42
2	109.91	5.00	1.58	0.12	5.75	11.32	63.15	524.06
3	58.85	5.00	1.03	0.09	7.75	14.00	97.22	1086.81
4	110.41	8.00	1.57	0.11	6.25	12.07	63.76	575.33
5	46.91	6.00	0.81	0.08	8.64	14.03	124.18	1547.26
6	43.84	5.00	0.67	0.1	6.72	12.36	149.91	1452.26
7	89.78	5.00	1.06	0.14	4.84	9.36	94.46	660.24
8	62.34	5.00	0.85	0.11	6.26	11.04	117.66	1061.91
9	101.12	4.00	1.32	0.12	5.93	10.78	75.65	647.13
10	29.30	5.00	0.63	0.07	9.74	16.78	159.87	2246.52
11	86.43	8.00	1.51	0.10	7.28	13.52	66.07	694.22
12	26.59	8.00	0.94	0.03	23.01	37.00	106.08	3521.84
13	28.03	5.00	0.53	0.09	7.75	14.19	187.61	2096.79
14	56.84	8.00	0.94	0.11	6.18	12.54	106.08	945.56
Mean	73.90	5.60	1.14	0.102	7.18	12.95	104.7	1245.47
SD	29.37	1.35	0.42	0.02	1.74	2.69	40.17	849.34
% CV	39.74	24.11	37.34	23.22	24.29	20.76	38.36	68.19

Table A6 Pharmacokinetic parameters of NDT after rectal administration of 100 mg tramadol suppository in healthy volunteers

Subjects	C_{max} (ng/mL)	T_{max} (h)	AUC $_{0-\infty}$ (µg.h/mL)	k_e (h^{-1})	$T_{1/2}$ (h)	MRT (h)	Cl/F (L/h)	Vz/F (L)
1	56.68	5.00	1.29	0.06	11.17	20.13	77.33	1252.37
2	13.20	5.00	0.23	0.06	10.72	17.80	434.59	6724.25
3	68.11	10.00	1.11	0.12	5.59	12.34	90.16	727.62
4	20.70	8.00	0.29	0.1	6.71	12.78	337.77	3267.79
5	8.96	6.00	0.11	0.16	4.38	9.23	890.34	5624.9
6	17.82	6.00	0.32	0.08	8.87	15.39	313.93	4016.81
7	20.01	6.00	0.28	0.11	6.48	11.76	359.07	3358.48
8	9.27	5.00	0.15	0.08	8.44	15.30	688.53	8388.37
9	8.86	6.00	0.097	0.12	6.01	11.61	1026.83	8897.44
10	33.41	10.00	0.88	0.05	13.32	22.89	112.90	2169.84
11	16.90	8.00	0.26	0.11	6.08	12.19	387.17	3395.13
12	27.28	8.00	0.47	0.09	7.82	15.63	214.53	2419.64
13	11.15	6.00	0.13	0.12	5.93	12.20	748.51	6408.60
14	9.23	8.00	0.49	0.38	1.80	7.99	2018.9	5247.98
Mean	25.70	6.70	0.47	0.09	8.17	14.92	550.07	4421.37
SD	20.89	1.95	0.44	0.03	2.85	4.27	516.99	2535.84
% CV	81.27	29.05	93.11	34.8	34.93	28.59	93.99	57.35

Table A7 Lab screening data of participants (n=14)

Lab	Reference range	Volunteers						
Hematology		TR01	TR02	TR03	TR04	TR05	TR06	TR07
Hb/Hct (gm%)	12-16/35-50	11.5/35.8	13.6/40.3	12.9/39.3	13.8/38.5	14.9/38.5	15.3/46.4	14.6/43.3
WBC (10^9/µl)	4.5-11.0	5.65	6.83	9.95	12	7.26	7.24	6.96
Neutrophil (%)	35-66	49.9	55	61.2	70.1	57	56.7	43
Lymphocyte (%)	24-44	42.8	33.1	33.8	21.5	36	30.9	47
Monocyte (%)	3-6	5	9.4	3.8	6.5	4.8	7.9	7
Eosinophil (%)	0-3	1.9	2.2	0.9	1.8	1.8	4.4	1
Basophil (%)	0-1	0.4	0.3	0.3	0.3	0.4	0.1	
Platelet (10^9/µl) (cell/cu.mm)	150-450	336	255	341	249	279	271	259
RBC (10^6 / µl)	4.2-5.5	4.01	4.54	4.23	4.7	5.1	5.66	5.04
MCV (fl)	83-97	89.3	88.8	92.9	82.6	84.5	82	85.9
MCH (pg)	27-33	28.7	30	30.5	29.5	29.2	27	29
MCHC (g/dl)	31-35	32.1	33.7	32.8	35.8	34.6	33	33.7

Table A7 Lab screening data of participants (n=14) (continued)

Lab	Reference range	Volunteers						
Hematology		TR08	TR09	TR10	TR11	TR12	TR13	TR14
Hb/Hct (gm%)	12-16/35-50	12-16/35-50	14.5/43.6	15.7/43.5	14.9/43.7	15.4/44.5	14.4/42.3	13.3/43.7
WBC (10^9/µl)	4.5-11.0	4.5-11.0	5.1	9.17	6.29	5.76	5.3	6.5
Neutrophil (%)	35-66	35-66	49.8	60.6	47	59.4	49	58.5
Lymphocyte (%)	24-44	24-44	36.5	32	45	33.7	36	24.1
Monocyte (%)	3-6	3-6	5.9	4.0	3	5.7	7	10.7
Eosinophil (%)	0-3	0-3	7.6	3.2	4	0.9	5	4.7
Basophil (%)	0-1	0-1	0.2	0.2	1	0.3	2	2
Platelet (10^9/µl) (cell/cu.mm)	150-450	150-450	186	259	263	209	296	222
RBC (10^6 / µl)	4.2-5.5	4.2-5.5	5.34	5.27	5.06	5.35	4.66	7
MCV (fl)	83-97	83-97	81.6	82.5	86.4	83.2	90.8	62.3
MCH (pg)	27-33	27.2	29.8	29.4	28.8	30.9	18.9	28.9
MCHC (g/dl)	31-35	33.3	36.1	34.1	34.6	34	30.4	33.7

Table A7 Lab screening data of participants (n=14) (continued)

Lab	Reference range	TR01	TR02	TR03	TR04	TR05	TR06	TR07
RDW (%)	11-16	15.9	12.4	12.0	10.7	12.7	13.1	12.2
eGFR-EP /eGFR-MD (ml/min/1.7)		126/119	125/114	126/115	125/116	125/120	128/127	129/131
Chemistry								
BUN (mg/dL)	5-20	11.2	10.7	9.8	15	7.1	12.6	11.2
Cr (mg/dL)	0.5-1.4	0.66	0.7	0.7	0.68	0.87	0.83	0.80
TB/DB (mg%)	0.1-1.2/0-0.3	0.39	0.5	1.04	0.23	0.31/0.06	0.46	0.56
AST (U/L)	20-48	20	18	21	22	16	21	21
ALT (U/L)	10-35	14	19	19	14	14	25	26
Alk phos (U/L)	51-153	65	74	52	76	95	74	86
Glu (mg %)	70-110	95	89	96	92	93	92	96
Anti-HIV screening		NR	NR	NR	NR	NR	NR	NR

Table A7 Lab screening data of participants (n=14) (continued)

Lab	Reference range	TR08	TR09	TR10	TR11	TR12	TR13	TR14
RDW (%)	11-16	13.6	11.8	11.6	11.5	12.5	12.1	12.3
eGFR-EP /eGFR-MD (ml/min/1.7)		129/133	127/126	122/115	112/105	128/129	124/120	103/97
Chemistry								
BUN (mg/dL)	5-20	11.1	9.4	8.9	16.4	7.9	11.3	12.7
Cr (mg/dL)	0.5-1.4	0.79	0.83	0.88	0.97	0.81	0.86	1.03
TB/DB (mg%)	0.1-1.2/0-0.3	0.49	1.01	0.7	1.17	0.75	0.63	0.75
AST (U/L)	20-48	19	30	18	15	13	17	23
ALT (U/L)	10-35	25	31	13	13	10	10	15
Alk phos (U/L)	51-153	61	85	58	60	82	81	31
Glu (mg %)	70-110	109	98	90	90	100	84	80
Anti-HIV screening		NR	NR	NR	NR	NR	NR	NR

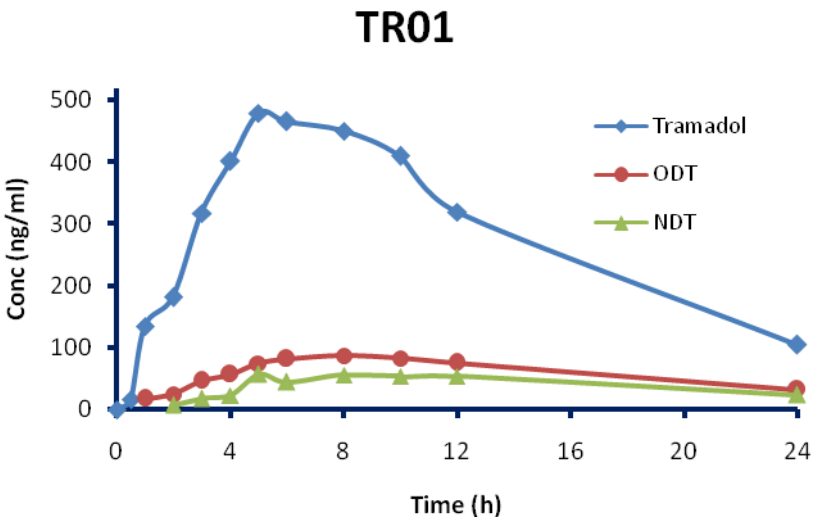

Figure A1 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR01 after administration of tramadol suppository 100 mg.

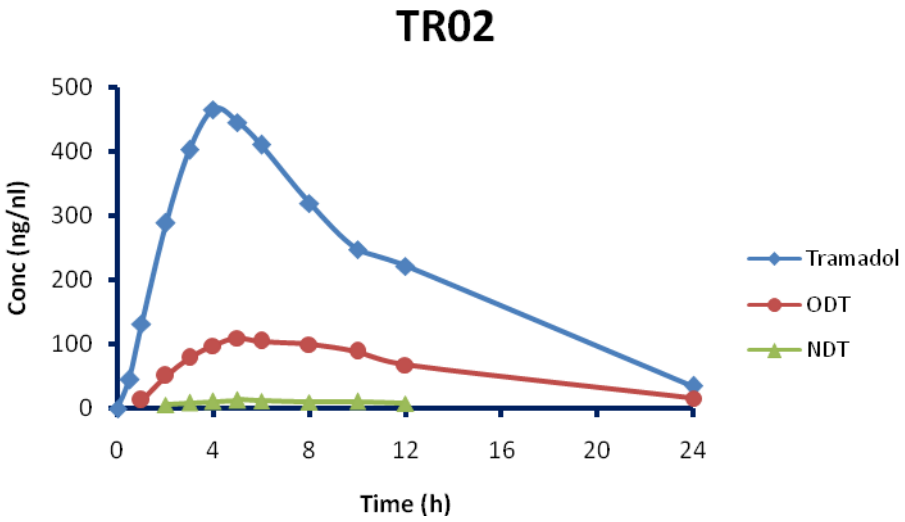

Figure A2 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR02 after administration of tramadol suppository 100 mg.

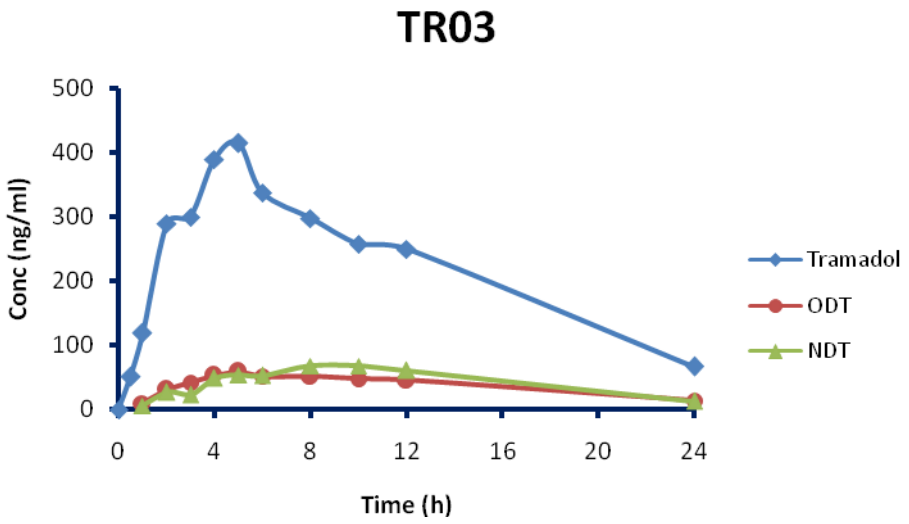

Figure A3 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR03 after administration of tramadol suppository 100 mg.

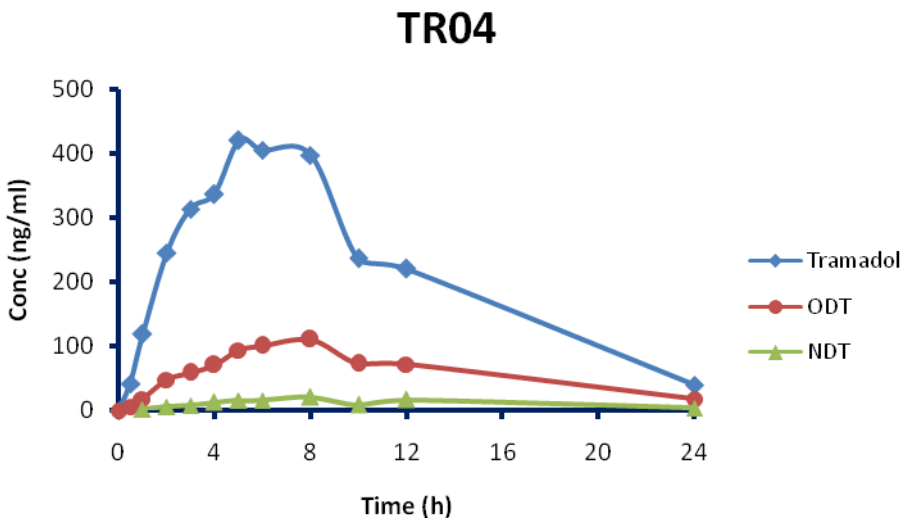

Figure A4 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR04 after administration of tramadol suppository 100 mg.

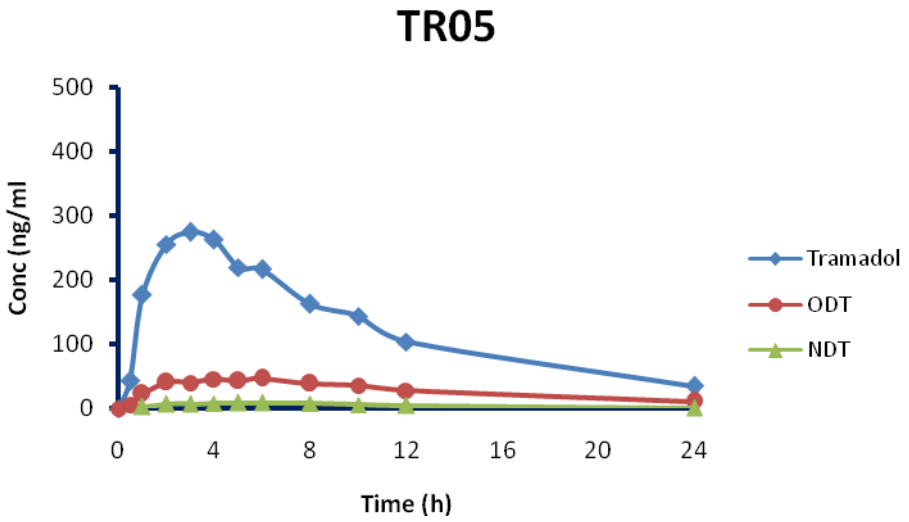

Figure A5 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR05 after administration of tramadol suppository 100 mg.

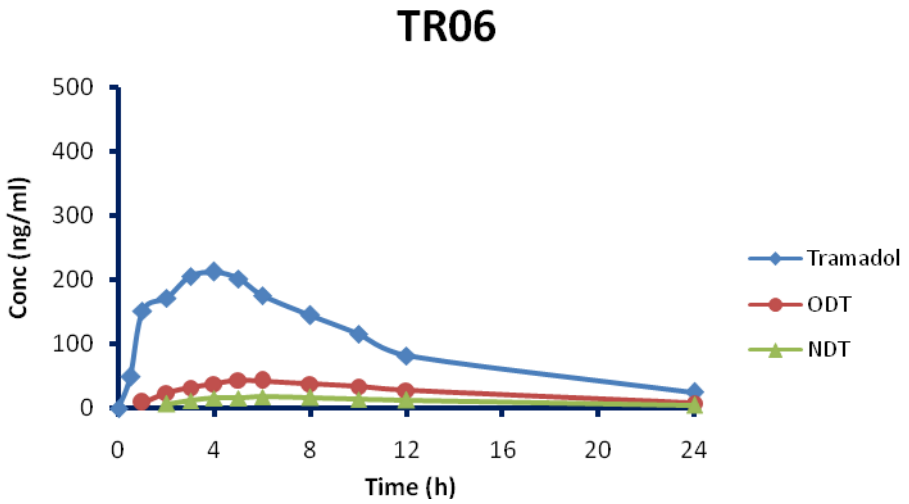

Figure A6 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR06 after administration of tramadol suppository 100 mg.

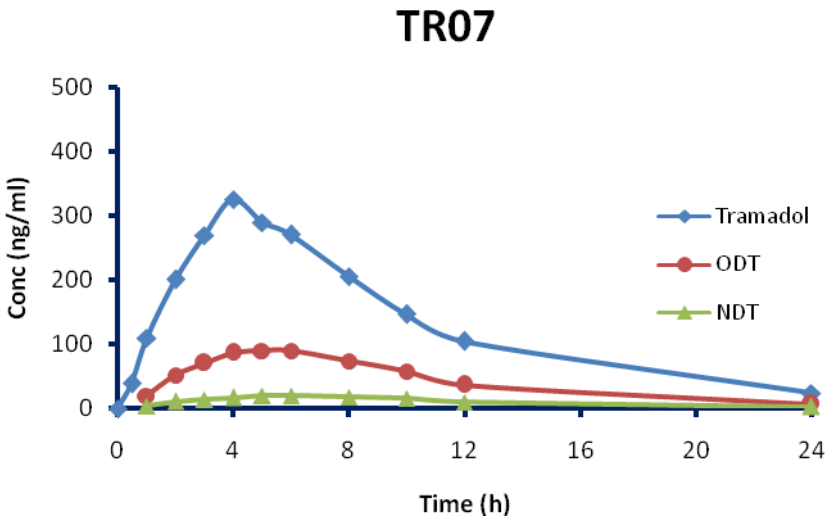

Figure A7 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR07 after administration of tramadol suppository 100 mg.

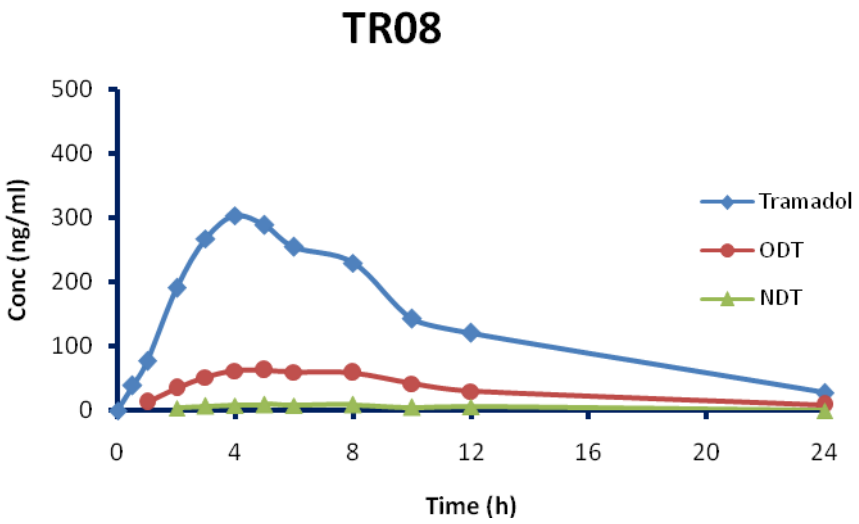

Figure A8 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR08 after administration of tramadol suppository 100 mg.

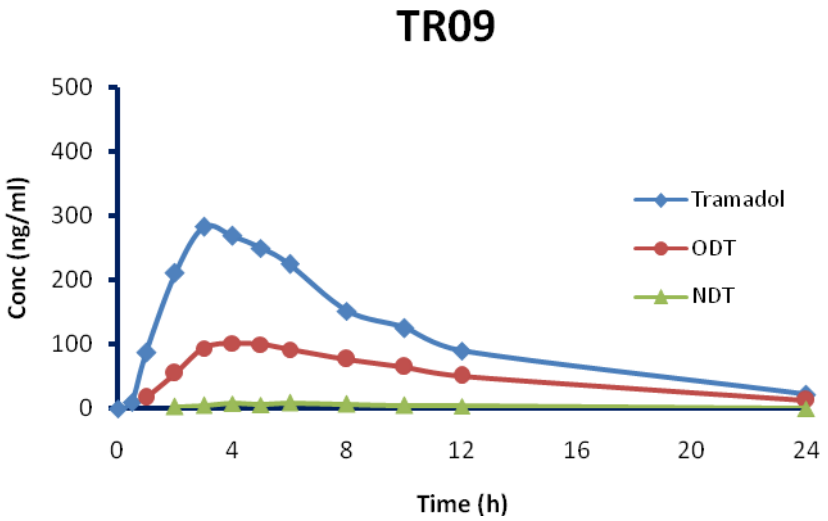

Figure A9 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR09 after administration of tramadol suppository 100 mg.

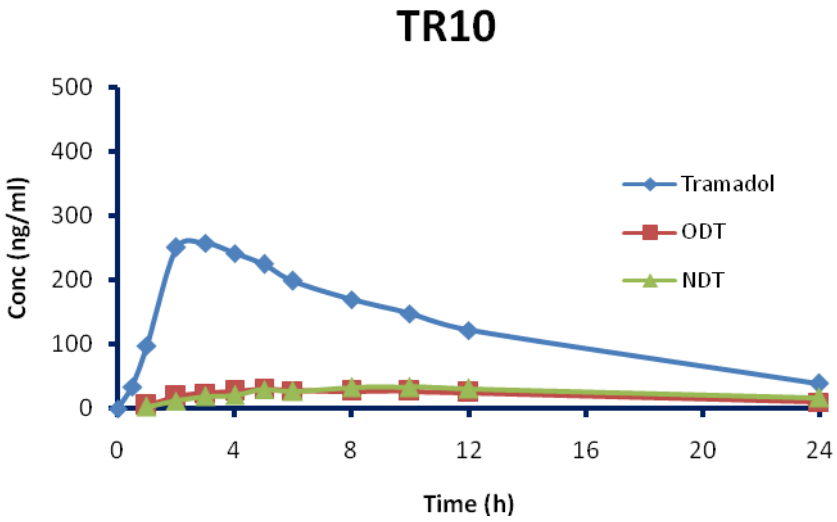

Figure A10 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR10 after administration of tramadol suppository 100 mg.

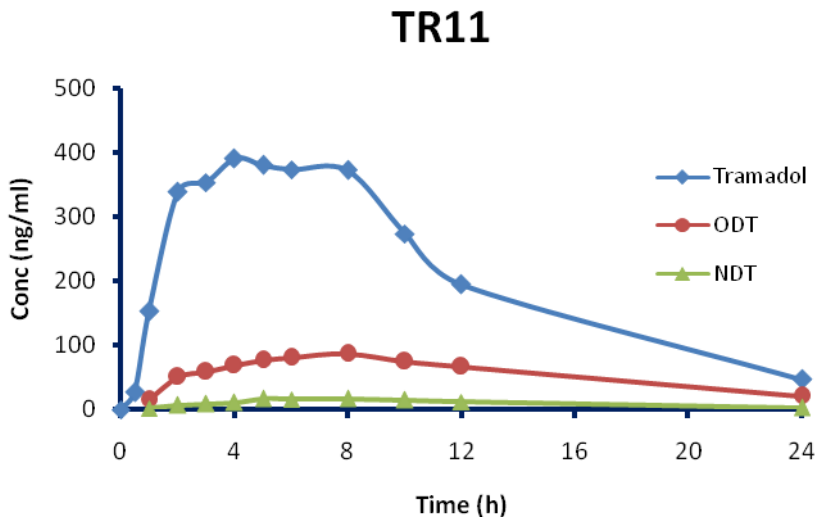

Figure A11 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR11 after administration of tramadol suppository 100 mg.

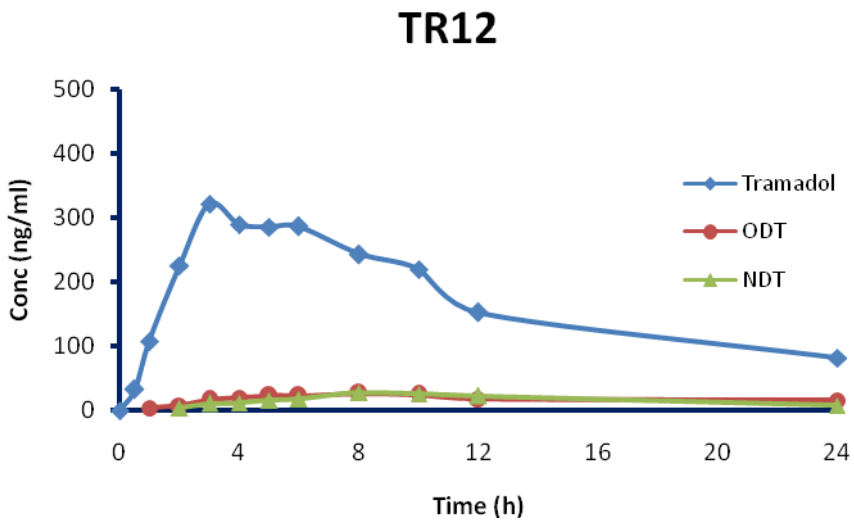

Figure A12 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR12 after administration of tramadol suppository 100 mg.

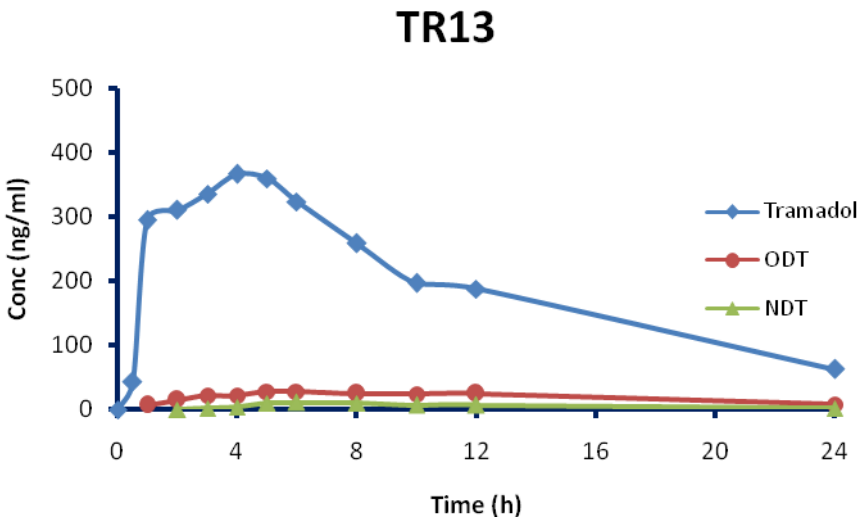

Figure A13 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR13 after administration of tramadol suppository 100 mg.

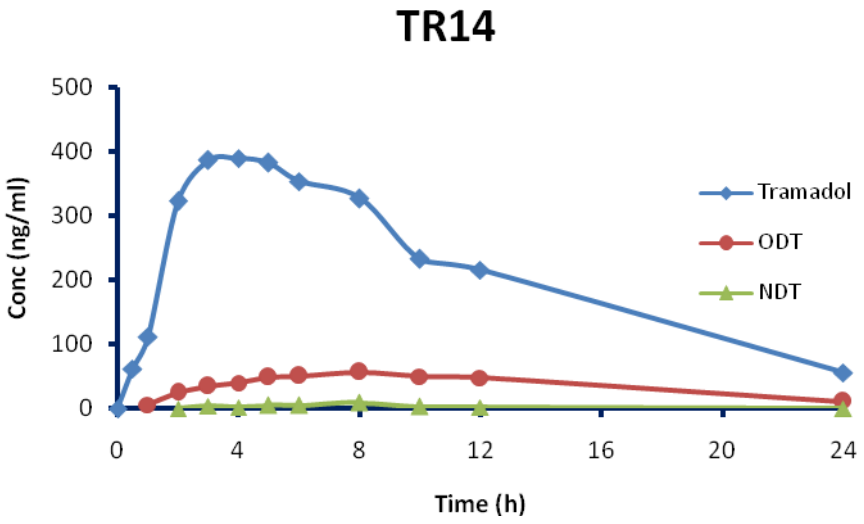

Figure A14 Plasma concentration time profile of tramadol, ODT and NDT of volunteer TR14 after administration of tramadol suppository 100 mg.

VITAE

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2. Education fee grant, Discipline of Excellence in Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Had yai, Thailand.

List of Proceeding

Shakya, S.R., Kaewnopparat, N. and Wiwattanawongsa K. In vitro evaluation of tramadol rectal suppositories. The 2nd ASEAN plus three Graduate Research Congress. February 5-7, 2014. Bangkok, Thailand

Shakya, S. R., Kaewnopparat, N. and Wiwattanawongsa K. In vitro release of tramadol from sustained release suppositories. The 2nd Current Drug Development International Conference. May 2-4, 2012. Phuket, Thailand