การศึกษาเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนต่อเมทแอมเฟตามีนใน ตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย

นางสาวนันทิกา แก้วปัญญา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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COMPARATIVE STUDY OF THE RATIO OF AMPHETAMINE TO METHAMPHETAMINE CONCENTRATION IN THE URINE SAMPLES OF THAI PATIENTS RECEIVING SELEGILINE AND METHAMPHETAMINE ABUSERS

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Thesis Title	COMPARATIVE STUDY OF THE RATIO OF AMPHETAMINE TO METHAMPHETAMINE CONCENTRATION IN THE URINE SAMPLES OF THAI PATIENTS RECEIVING SELEGILINE AND METHAMPHETAMINE ABUSERS
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นันทิกา แก้วปัญญา : การศึกษาเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนต่อเมทแอมเฟตามีนในตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย (COMPARATIVE STUDY OF THE RATIO OF AMPHETAMINE TO METHAMPHETAMINE CONCENTRATION IN THE URINE SAMPLES OF THAI PATIENTS RECEIVING SELEGILINE AND METHAMPHETAMINE ABUSERS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. พ.ต.ท.หญิง ดร.สมทรง ลาวัณย์ประเสริฐ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : นพ.อัครวุฒิ วิริยเวชกุล, พ.ต.ท.วิเชียร ตั้งธนานุวัฒน์, 80 หน้า.

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย และประเมินความเป็นไปได้ในการนำค่าอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนมาใช้เป็นข้อมูลเบื้องต้นในการประเมินแยกผู้ป่วยที่ได้รับยาซีลีจิลีนออกจากผู้ติดยาเสพยาเสพติดชนิดเมทแอมเฟตามีน เก็บตัวอย่างปัสสาวะจากผู้ป่วยชาวไทย 15 ราย (ชาย 11 ราย และ หญิง 4 ราย, อายุ 45-76 ปี) ซึ่งเป็นผู้ป่วยนอกของสถาบันประสาทวิทยาและได้รับยาซีลีจิลีนในขนาดการรักษา ตัวอย่างปัสสาวะถูกเก็บที่เวลา 2, 4, 6, 8 และ 20 ชั่วโมงหลังจากรับประทานยาซีลีจิลีน ตัวอย่างปัสสาวะจากผู้ติดยาเสพติด 97 รายถูกเก็บคนละหนึ่งครั้งที่เวลา 2, 4, 6, 8 หรือ 20 ชั่วโมงหลังเสพยาเสพติดชนิดเมทแอมเฟตามีนครั้งสุดท้าย ทำการวิเคราะห์ความเข้มข้นของเมทแอมฟตามีนและแอมเฟตามีนในตัวอย่างปัสสาวะด้วยวิธี solid-phase microextraction-gas chromatography-mass spectrometry

อัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนที่วิเคราะห์ได้ในปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีนมีค่าที่สูงกว่าผู้ติดยาเสพติดอย่างมีนัยสำคัญทางสถิติ (P<0.01) ที่เวลา 2, 4, 6, 8, และ 20 ชั่วโมงหลังจากที่ผู้ป่วยได้รับยาซีลีจิลีนหรือจากหลังจากการเสพยาครั้งสุดท้ายในผู้ติดยาเสพติด โดยค่าต่ำสุดที่พบในผู้ป่วยคือ 0.74 ± 0.07 ในขณะที่ค่าสูงสุดในผู้ติดยาเสพติดคือ 0.41 ± 0.05 ที่เวลา 6 ชั่วโมง ค่าอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนสามารถนำมาใช้เป็นข้อมูลเบื้องต้นในประเมินแยกผู้ป่วยที่ได้รับยาซีลีจิลีนออกจากผู้เสพยาเสพติดชนิดเมทแอมเฟตามีน โดยมีความถูกต้อง 84.88% เมื่อใช้ค่าอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีน 0.4 เป็นจุดตัดในการแยกผู้ป่วยออกจากผู้ติดยาเสพติด

ภาควิชา เภสัชวิทยาและสรีรวิทยา ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชวิทยา ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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NUNTHIKA KAEWPUNYA : COMPARATIVE STUDY OF THE RATIO OF AMPHETAMINE TO METHAMPHETAMINE CONCENTRATION IN THE URINE SAMPLES OF THAI PATIENTS RECEIVING SELEGILINE AND METHAMPHETAMINE ABUSERS. ADVISOR : ASSOC. PROF. POL. LT. COL. SOMSONG LAWANPRASERT, PH. D., CO-ADVISOR : AKRAVUDH VIRIYAVEJAKUL, M.D., POL. LT. COL. WICHIAN TUNGTANANUWAT, 80 pp.

The objective of this study was to compare the ratio of amphetamine to methamphetamine concentrations in urines of Thai patients receiving selegiline therapy to the ratio of amphetamine to methamphetamine concentrations in urines of Thai methamphetamine abusers. In addition, the possibility of using the ratio of amphetamine to methamphetamine to preliminarily differentiate patients receiving selegiline therapy from methamphetamine abusers was also assessed. Urine samples were collected from fifteen Thai patients (11 men and 4 women, 45-76 years old). They were outpatients of Prasat Neurological Institute and were prescribed selegiline for medical purpose. Urine samples were collected at 2, 4, 6, 8 and 20 hours after selegiline administration. The urine samples from 97 abusers were collected once from each abuser at either 2, 4, 6, 8, or 20 hours after the last methamphetamine consumption. Methamphetamine and amphetamine concentrations in urine samples were determined using solid-phase microextraction-gas chromatography-mass spectrometry.

The ratio of amphetamine to methamphetamine concentrations in urines of patients receiving selegiline were significantly (p<0.01) higher than those of the methamphetamine abusers at every corresponding time point (2, 4, 6, 8 and 20 hours) after selegiline administration or methamphetamine consumption. The lowest ratio of the patients was 0.74 ± 0.07, while the highest ratio of the methamphetamine abusers was 0.41 ± 0.05 at 6 hours. The ratio of amphetamine to methamphetamine concentrations in urines could be preliminarily used to differentiate patients receiving selegiline from methamphetamine abusers with an accuracy of 84.88% when using the value ratio of 0.4 as the cut-off value.

Department : Pharmacology and Physiology Student's Signature .....

Field of Study : Pharmacology Advisor's Signature .....

Academic Year : 2011 Co-advisor's Signature .....

Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

α	=	alpha
AM	=	amphetamine
β	=	beta
CNS	=	central nervous system
(R^2)	=	coefficient of determination
r	=	correlation coefficient
°C	=	degree of Celsius
DA	=	dopamine
DAT	=	dopamine transporter
d- AM	=	*dextrorotary* -amphetamine
d- MA	=	*dextrorotary*-methamphetamine
et al.	=	et alii (and others)
γ	=	gamma
GC-MS	=	gas chromatography-mass spectrometry
l-AM	=	*levorotatory*- amphetamine
l-MA	=	*levorotatory*-methamphetamine
LC-MS	=	liquid chromatography-mass spectrometry
MA	=	methamphetamine
µg/l	=	microgram per litre
µl	=	microlitre
mg	=	milligram
ml	=	millilitre
MAO	=	monoamine oxidase
NA	=	noradrenaline
NET	=	noradrenaline transporter
/	=	per
PE	=	β-phenylethylamine
KOH	=	potassium hydroxide

n	=	sample size
5HT	=	serotonin
SERT	=	serotonin transporter
NaCl	=	sodium chloride
SD	=	standard deviation
SEM	=	standard error of the mean
TPC	=	(*S*)-(—)-*N*-(trifluoroacetyl)-propyl chloride
VMAT-2	=	vesicular monoamine transporter-2

CHAPTER I

INTRODUCTION

Methamphetamine (MA) is presently one of the most popular illicit drugs world-wide including Thailand (Sherman, 2007). MA and amphetamine (AM) of the (*d*-) form or (+) isomer, are more frequently abused because they possess stronger psychostimulating activity than the corresponding (*l*-) form or (—)-enantiomer (Chiang, 1990). Both MA and AM are classified as type I narcotic drugs according to the Thai Narcotic Act B.E. 2522. Use of MA is normally detected by determination of MA and its metabolite, AM in urine. Any persons with urine MA or AM concentrations of $\geq$ 1000 ng/ml are accused as illegal MA or AM consumption. Thus, urine concentration of these substances of $\geq$ 1000 ng/ml is used as the cut-off value for positive interpretation of illicit abuser of MA or derivatives of AM according to the regulation.

Selegiline is a selective irreversible monoamine oxidase B inhibitor used in the treatment of Parkinson's disease in combination with levodopa (Hardman et al., 1996). After administration, selegiline is rapidly metabolized in the liver via two reactions; (1) *N*-desmethylation yielding desmethylselegiline which is further metabolized to (*R*)-(—)-amphetamine by *N*-despropynylation, (2) *N*-despropynylation yielding (*R*)-(—)-methamphetamine which is further metabolized to (*R*)-(—)-amphetamine by *N*-desmethylation. Both (*R*)-(—)-methamphetamine and (*R*)-(—)-amphetamine are further converted to other minor metabolites by *p*-hydroxylation and β-hydroxylation. Thus, 9 metabolites of selegiline were found in urine as following; desmethylselegiline, (*R*)-(—)-methamphetamine, (*R*)-(—)-amphetamine, (1*S*, 2*R*)-norephedrine, (1*R*, 2*R*)-norpseudoephedrine, (1*S*, 2*R*)-(+)-ephedrine, (1*R*, 2*R*)-(—)-pseudoephedrine, (*R*)-(—)-*p*-hydroxyamphetamine, (*R*)-(—)-*p*-hydroxymethamphetamine as well as selegiline which is excreted as an unchanged drug. Within 24 hours after selegiline administration, the major metabolite found in urine is MA while AM is found with a lesser amount (Shin, 1997).

Since the majority of selegiline metabolites in urine is (—)-MA or *l*-AM with a lesser amount of (—)-AM or *l*-AM, false positive interpretation of patients receiving selegiline as MA abusers could occur based on the routine forensic toxicological analysis. This could occur eventhough MA and AM in urines of MA abusers are found as dextrorotary (*d*-) forms (Baselt,

2002) whereas MA and AM which are also the metabolites of selegiline are found as the levorotatory (*l*-) form (Baselt, 2002). To differentiate patients receiving selegiline from MA abusers, analysis of MA and AM in urines must be able to differentiate the compounds stereospecifically which is not normally performed in the routine analyses.

Detection of MA and AM in urine samples in forensic toxicological analysis is generally divided into 2 processes. Firstly, preliminary screening test is performed using color test or immunoassay. The sample with positive result is further confirmed by confirmatory test based on chromatographic technique such as thin layer chromatography, gas chromatography, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Due to the limitation of the routine forensic toxicological analysis to differentiate the compounds with enantiomers, enantioselective and sufficiently sensitive methods of determination have been developed such as utilization of derivatizing reagents (Chang et al., 2001; Chiu et al., 2004; Tzing et al., 2006) or chiral column (Hasegawa, 1999) during the analysis using GC-MS (Frank et al., 1978; Konig and Benecke, 1981; Liu et al., 1982; Hasegawa, 1999; Chang et al., 2001; Chiu et al., 2004; Tzing et al., 2006) or LC-MS (Wainer and Doyle, 1983; Armstrong, 1987; Karnes and Sarkar, 1987; Pirkle et al., 1987).

Several previous studies determined the concentrations of (*l*-)-MA and (*l*-)-AM in urine samples collected from dead bodies and patients receiving high doses of selegiline. They found that the ratios of AM/MA concentrations were 0.3 (Meeker and Reynolds, 1990), 0.46 (Kupice and Chaturvedi, 1999), 0.33 (Kim et al., 2000) and 0.40 (Fujita et al., 2008), while the ratio of AM/MA concentrations in urines of MA abusers were less than 0.20 (Kim et al., 2000). Hasegawa et al. (1999) reported that the ratio of AM/MA gradually increased from 0.24 to 0.67 ($r = 0.857$) from 2-48 hours after selegiline administration. In contrast, the ratio of AM/MA was less than 0.24 in 74% of the 50 MA abusers. These groups of researchers suggested that the ratio of urinary AM/MA concentrations may be useful to distinguish patients receiving selegiline from MA abusers before performing the confirmation test which is capable to differentiate the stereoisomer compounds.

The previous studies mentioned above were performed using urines of healthy volunteers, patients and MA abusers in Western countries and Japan. No studies have been reported in Thai patients receiving selegiline at clinically therapeutic dose and in Thai MA

abusers. Metabolism of selegiline occurs in the liver using CYP2B6, CYP2C19 and CYP2D6 (Hidestrand et al., 2001; Torre et al., 2004; Benetton et al., 2007). Methamphetamine is metabolized to amphetamine by CYP2D6 (Cruickshcnk and Dyer, 2009). These CYP isoforms have been known to possess polymorphism (Kraemer and Maurer, 2002) which is genetically affected by race that may influence the ratio of AM/MA concentrations in urines. Thus, the objective of this study is to determine the comparative ratio of AM to MA concentrations in the urine samples of Thai patient receiving selegiline therapy compared to that of MA abusers. The ratio of AM/MA concentrations was tested so as to assess the possibility of using this ratio to differentiate patients receiving selegiline from MA abusers.

Hypothesis

The ratio of AM/MA concentrations in urines of patients receiving selegiline therapy was significantly different from the ratio of AM/MA concentrations in urines of MA abusers. The ratio of AM/MA could be used to differentiate patients receiving selegiline from MA abusers.

Objectives

1) To compare the ratio of AM/MA concentrations in urines Thai patients receiving selegiline therapy to the ratio of AM/MA concentrations in urines Thai MA abusers.
2) To assess the possibility of using the ratio of AM/MA to preliminarily differentiate patients receiving selegiline therapy from MA abusers.

Benefit gained from the study

Result from this study provides the ratio of AM/MA concentrations in urines of Thai patients receiving selegiline therapy and the ratio of AM/MA concentrations in urines of Thai MA abusers. The ratio of AM/MA could be preliminarily used to differentiate patients receiving selegiline from MA abusers.

CHAPTER II

LITERATURE REVIEW

Selegiline

Selegiline (*l*-deprenyl) is an irreversible inhibitor of monoamine oxidase (MAO) type B. Selegiline increases dopamine in the central nervous system because dopamine is mainly metabolized by MAO-B. Selegiline also inhibits presynaptic uptake dopamine and noradrenaline. Thus, selegiline is presently used in the treatment of Parkinson's disease either selegiline alone or in combination with levodopa (Heinonen and Lammintausta, 1991).

A. Chemical properties

The chemical name is (α*R*)-*N*, α-Dimethyl-*N*-2-propynylbenzeneethanamine; L-(—)-*N*, α-dimethyl-*N*-2-propynylphenethylamine; (—)-deprenil; L-deprenyl. It has a molecular weight of 187.28 (The Merck Index, 2006). Its chemical structure is shown in Figure 1.

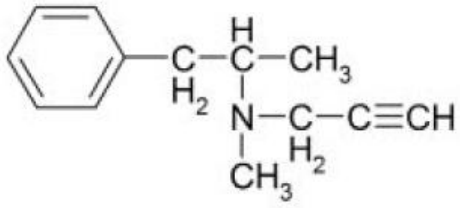


Figure 1 The chemical structure of selegiline (Eric and Rachel, 2008)

B. Pharmacology

1. Mechanism of action (Heinonen and Lammintausta, 1991)

Selegiline inhibits the activity of monoamine oxidase (MAO) by a "suicide reaction", initially competitive and reversible interaction between an inhibitor and the enzyme followed by the formation of an irreversible adduct. MAO contains 8-alfa-cysteinyl-FAD (flavin adenine dinucleotide), which seems to be the site for the irreversible bonding between the enzyme and the inhibitor (Youdim, 1978). Selegiline selectively inhibits MAO-B at low dose but also inhibit MAO-A at high dose (Knoll, 1978). Action of selegiline on MAO-B causing an enhancement of the dopaminergic transmission in the brain (Figure 2). Selegiline have been used successfully in

the treatment Parkinson's disease which the main pathophysiological finding is the destruction of the substantia nigra leading to dopamine deficiency at striatum.

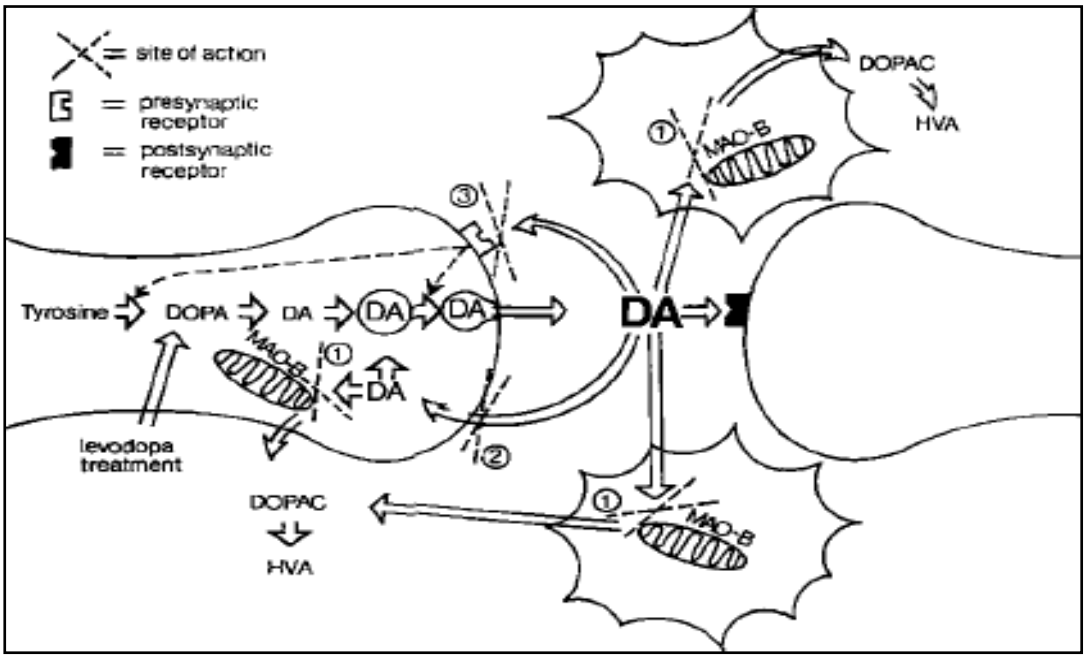


Figure 2 Modes of action of selegiline to increase concentration of dopamine in synaptic cleft: 1. inhibition of MAO-B, 2. inhibition of reuptake, 3. inhibition of presynaptic autoreceptor (DA= dopamine, DOPAC = 3,4 dihydroxyphenylaceti acid, HVA = homovanillic acid) (Heinonen and Lammintausta, 1991).

MAO is located in the outer membrane of the mitochondrion. It principally inactivates monoamine transmitters and other monoamines in both the central nervous system (CNS) and in peripheral neurons. MAOs are currently subclassified into type A and type B, which differ in their substrate preferences and sensitivity to inhibition by MAO inhibitor, Clorgyline (Johnston, 1968). Distribution of MAO-A and MAO-B in man and other species is shown in Table 1. In gastrointestinal tract, MAO is predominantly type A (MAO-A). This enzyme metabolizes the oxidation of tyramine, but in human CNS, it is responsible for the deamination of serotonin (5-hydroxytryptamine; 5HT) and noradrenaline (NA). While the MAO-B plays a role principally in the catabolism of dopamine (DA) and β-phenylethylamine (PE) (Foley et al., 2000).

Table 1 Distribution of MAO-A and MAO-B in man and in the brains of selected species (Foley et al., 2000)

Tissue (man)	% of total activity	
	MAO-A	MAO-B
Liver	45	55
Gastrointestinal tract	< 80	> 20
Kidneys	25	75
Lungs	55	45
Platelets	< 5	> 95
Brain:		
Human	< 20	> 80
Guinea pig	20	80
Cat	25	75
Pig	40	60
Rat	55	45

2. Therapeutic use

Selegiline is an irreversible MAO-B inhibitor used in the treatment of Parkinson's disease, both as monotherapy and in combination with levodopa (Hardman et al., 1996). Although levodopa is the most effective for Parkinson's disease therapy, majority of patients experience motor fluctuations, dyskinesia and other compliance after 5 years or even during the first year of levodopa therapy. Neurotoxicity could possibly be the other reason for delaying levodopa therapy. Levodopa therapy is thus usually indicated when treatment with selegiline, anticholinergics such as amantadine or DA agonists are no longer provide satatisfactory control of the symptoms. Selegiline is well tolerated at the usual dose but it occasionally causes nausea, insomnia and hallucinations.

Using in Parkinson's disease, selegiline at 5-10 mg daily can prolong and potentiate the efficacy of levodopa. Levodopa dose can be reduced and fluctuations in clinical disability related

to dosing can be reduced. Use of the drug alone in the early phase of the disease can delay the initiation of levodopa therapy. (Heinonen and Lammintuste, 1991).

Administration of selegiline at 10 mg daily dose, MAO activity in human brain towards DA was inhibited by 90% and towards 5-HT by about 65% (Riederer et al,. 1978). After a single dose of 5 mg of selegiline, about 90% of platelet MAO-B is inhibited within 4 hours after administration. Furthermore after 10 mg of selegiline administration, platelet MAO-B is almost completely inhibited within 24 hours. The rate and degree of platelet MAO-inhibition is similar either after 10 mg once daily in the morning or 5 mg twice daily (morning and noon) (Lee et al., 1989).

C. Pharmacokinetic

1. Absorption

Selegiline is rapidly absorbed from the gastrointestinal tract, with peak plasma concentration usually attained within 0.5-2 hours after oral administration of a therapeutic dose (5-10 mg) (Foley et al., 2000). Bioavailability of the parent compound is about 10% due to considerable first pass metabolism (Heinonen et al., 1994). Administration selegiline with food increases amount of selegiline absorbed for about three times without changing the plasma concentration of its metabolism (Barrett et al., 1996).

2. Distribution and protein binding

Selegiline is distributed rapidly into the tissues including the brain**.** This must be due to the lipophilic property of the substance. The apparent volume of distribution is up to 1850 l. Ninety percent bound to plasma protein. Platelet MAO-B activity is inhibited 90% within 30-90 min in Parkinson patients. Recovery of MAO-B activity requires as long as 40 days (Fowler et al., 1994).

3. Metabolism

Selegiline is metabolized in the liver via the cytochrome P450 system. After administration, selegiline is rapidly metabolized in the liver via two reactions; (1) *N*-desmethylation yielding desmethylselegiline which is further metabolized to (*R*)-(—)-amphetamine by *N*-despropynylation, (2) *N*-despropynylation yielding (*R*)-(—)-methamphetamine which is further metabolized to (*R*)-(—)-amphetamine by *N*-desmethylation. Both (*R*)-(—)-methamphetamine and (*R*)-(—)-amphetamine are further converted to other minor

metabolites by *p*-hydroxylation and β-hydroxylation. Thus, 9 metabolites of selegiline were found in urine as following; desmethylselegiline, (*R*)-(—)-methamphetamine, (*R*)-(—)-amphetamine, (1*S*, 2*R*)-norephedrine, (1*R*, 2*R*)-norpseudoephedrine, (1*S*, 2*R*)-(+)-ephedrine, (1*R*, 2*R*)-(—)-pseudoephedrine, (*R*)-(—)-*p*-hydroxyamphetamine, (*R*)-(—)-*p*-hydroxymethamphetamine (Figure 3) as well as selegiline which is excreted as an unchanged drug. Within 24 hours after selegiline administration, the major metabolite found in urine is MA while AM is found with a lesser amount (Shin, 1997).

It is shown that several isoforms of hepatic cytochrome P450 (CYP) involve in the formation of the metabolites. Those isoforms include CYP2B6, CYP2C19, CYP1A2, CYP2C8 and CYP2D6 (Figure 4). Thus, patients with liver impairment or those receiving a drug that induces hepatic enzyme activity, their selegiline dosage adjustments are needed due to the pharmacokinetic change of selegiline (Anttila et al., 2005).

4. Elimination

Selegiline is eliminated by the kidney within 24 hours after selegiline administration. Urinary excretion of the three majority of metabolites was 1.1% of desmethylselegiline, 59.2% of (*R*)-(—)-methamphetamine and 26.3% of (*R*)-(—)-amphetamine (Heinonem et al., 1989). Half life of these metabolites were 3.4, 11.3 and 15.8 hours, respectively (Laine et al., 2000). Functions of liver and kidney affect elimination rate of selegiline and its metabolites. No significant change for desmethylselegiline in patients with kidney impair function. Half-lifes of *l*-MA and *l*-AM were 2 folds higher than those of the control group. In patient with drug-induced liver function, half-life of *l*-MA was higher than those of the control group. In contrast, *l*-AM was lower in patients as compared to the control group. The elimination rate of selegiline was substantially increased in subjects with drug-induced liver function and decreased in subjects with impaired liver function (Anttila et al., 2005). Urinary excretions of MA and AM depend largely on urinary pH. The excretion of MA and AM metabolites can be manipulated by marking urine either alkaline by giving the patients sodium bicarbonates or acidic by giving ammonium chloride (Elsworth et al., 1982).

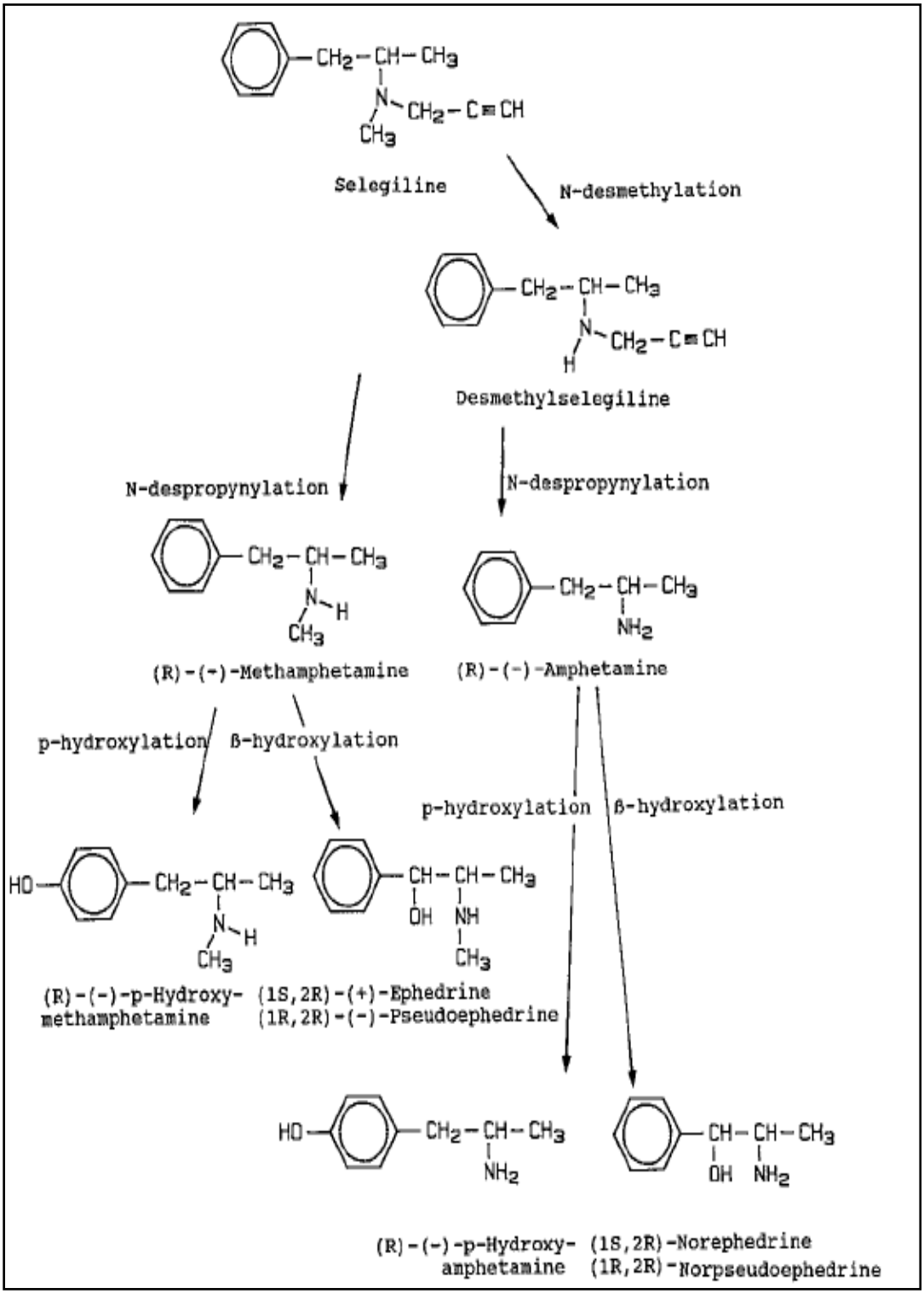


Figure 3 Metabolic pathways of selegiline in human (Shin, 1997)

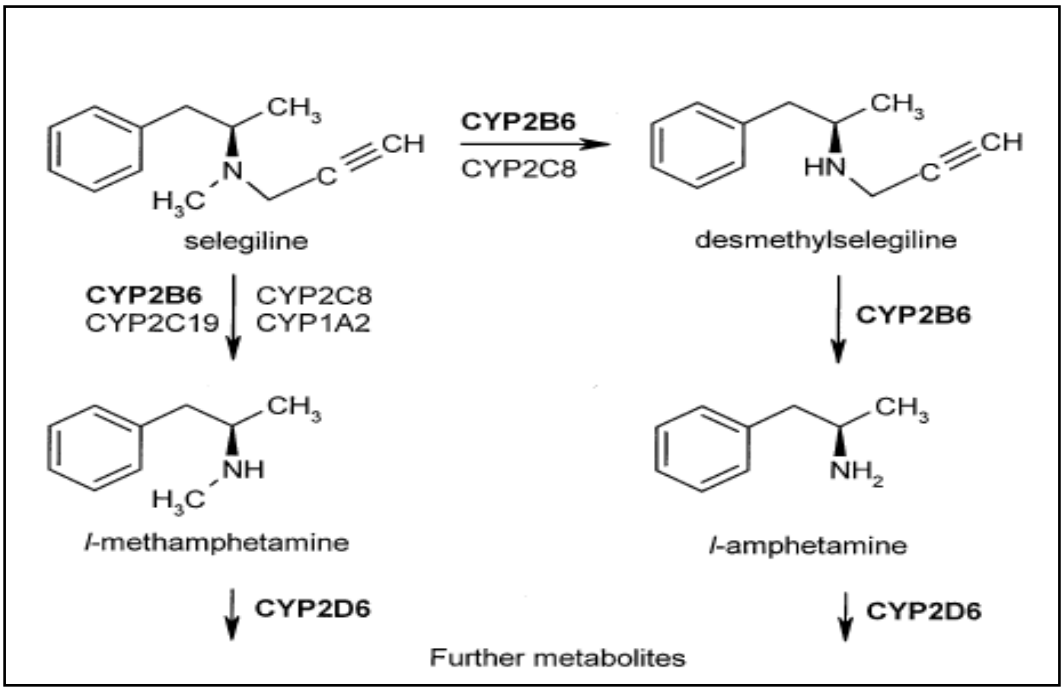


Figure 4 Proposed metabolic pathways of selegiline in humans and the enzyme cytochrome P450 involve in the reactions (Anttica et al., 2005)

Methamphetamine

Methamphetamine (MA) is a synthetic derivative of ephedrine. MA possesses stereogenic center, thus has two optically isomers. One is the *d*-(+)-methamphetamine which demonstrates stronger CNS stimulatory effect than the other which is *l*-(—)-enantiomer (Jirovsky et al., 1998). MA is presently one of the most popular illicit drug world-wide (United Nations Office on Drugs and Crime, 2007).

A. Chemical properties

The chemical name of methamphetamine is (α*S*)-*N*, α-Dimethylbenzeneethanamine; (*S*)-(+)-*N*, α-dimethylphenethylamine; *d*-*N*-methylamphetamine; *d*-deoxyephedrine; *d*-desoxyephedrine; l-phenyl-2-methylaminopropane; *d*-phenylisopropylmethylamine; methyl-*β*-phenylisopropylamine; Norodin with the chemical formula of $C_{10}H_{15}N$. Its molecular weight is 149.23 (The Merck Index, 2006). The chemical structure of MA is shown in Figure 5.

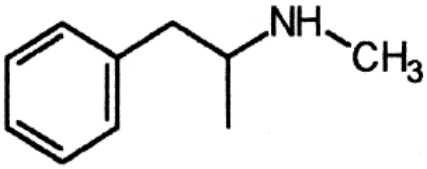


Figure 5 The chemical structure of methamphetamine (Jirovsky, 1998)

B. Pharmacology

1. Mechanism of action

MA is an indirect agonist at DA, NA and 5HT receptors. Because of the structural similarity, MA replaces monoamines at membrane-bound transporters such as dopamine transporter (DAT), noradrenaline transporter (NET), serotonin transporter (SERT) and vesicular monoamine transporter-2 (VMAT-2). MA replaces monoamines from storage vesicles into the cytosol and causing the release of DA, NA and 5HT from the cytosol into synapses. Synaptic monoamines are then available to stimulate postsynaptic monoamine receptors (Figure 6). In addition, MA attenuates monoamine metabolism by inhibiting monoamine oxidase (Sulzer et al., 2005; Cruickshank and Dyer, 2009).

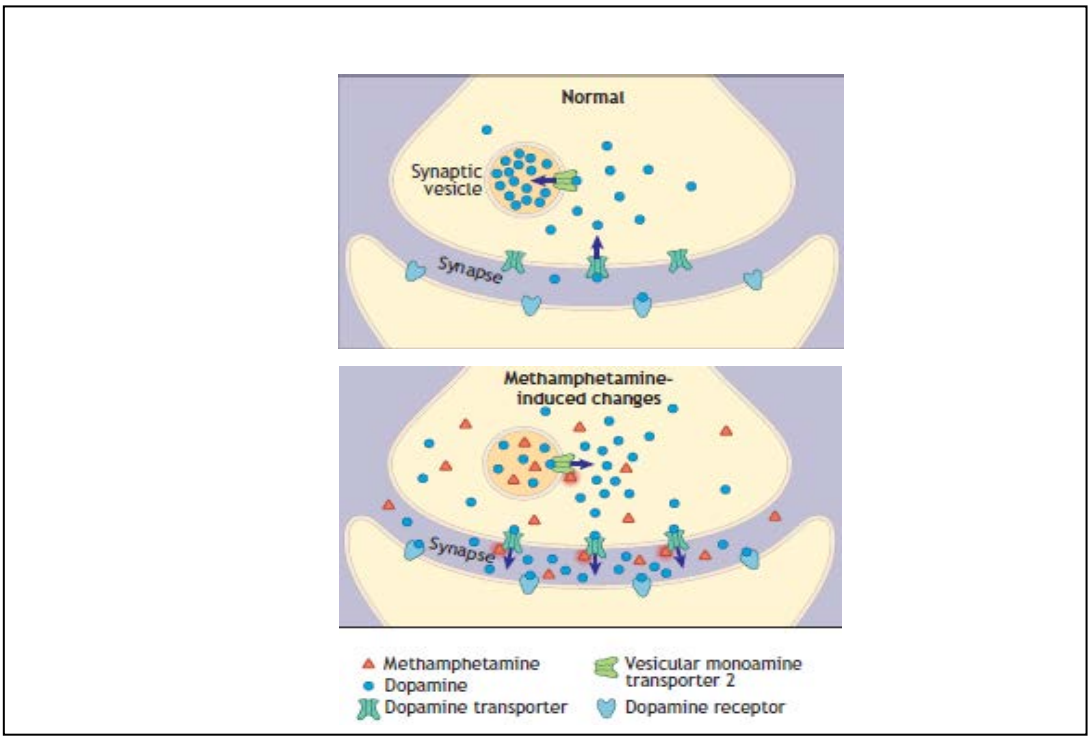


Figure 6 Mechanism of action of MA which causes the release of striatal dopamine from the nerve ending into the synapse (Stephen, 2008)

2. Therapeutic use

MA produces potent CNS mediated stimulant, anorectic and cardiovascular effect (Perez et al., 1991). In Thailand, it is classified as type I narcotic drugs according to the Thai Narcotic Act B.E. 2522. Oral MA is approved in the United States for the treatment of attention-deficit hyperactivity disorder in children and short-term treatment of exogenous obesity (Kish, 2008), while *l*-MA is clinically used in inhaler for nasal decongestant such as Vicks Inhaler, which does not possess any significant CNS activity or addiction (Hoffman and Lefkowitz, 1996). At the dose used in clinical experiment such as 5-30 mg, the prominent MA responses were arousal, reduced fatigue, euphoria, positive mood, accelerated heart rate, elevated blood pressure, pupil dilation, increased temperature, reduced appetite, behavioral disinhibition and short-term improvement in cognitive domains, relaxation, loss of tension, self-confidence, and anxiety (Cruickshank and Dyer, 2009).

C. Pharmacokinetic

1. Absorption and distribution

MA is available in many forms and can be smoked, snorted, injected, or orally ingested (Leshner, 1998). When MA is orally administered, it is rapidly absorbed from the gastrointestinal tract. MA is highly lipid soluble and readily cross the blood brain barrier. After oral ingestion of 30 mg MA, the average maximum peak plasma concentration is 94.1 μg/l which is reached at approximately 3 hours (Shappell et al., 1996).

2. Metabolism

MA is metabolized largely in the liver via three reactions: 1) *N*-demethylation resulting in amphetamine which is a major active metabolite 2) aromatic hydroxylation, producing primarily 4-hydroxymethamphetamine and 3) Further metabolism of amphetamine via deamination (yielding phenylacetone), aromatic hydroxylation (yielding 4-hydroxyamphetamine), β-hydroxylation (resulting in norephedrine) (Figure 7) (Moore, 1999). It is shown that CYP 2D6 involves in the reactions of *N*-demethylation and aromatic hydroxylation whereas CYP3A4 and CYP2B6 are also involved in the reaction of *N*-demethylation (Torre et al., 2004) (Figure 5).

3. Elimination

Approximately 70% of a MA dose is excreted in the urine within 24 hours; 40-50% as unchanged MA, 15% as 4-hydroxymethamphetamine and 4-7% as amphetamine (Moore K, 1999;

williams et al., 2000). Plasma half life is approximately 10 hours following 30 mg of MA similarly among administration routes (Table 2) (Cruickshank and Dyer, 2009).

The plasma half-life of MA and AM are mostly dependent on the acidity of the urine. Since MA and AM are weak basic substances, renal excretion is increased by urinary acidification and decreased by urinary alkalinisation (Quinn et al., 1997). In addition, repeated doses are related to an accumulation of MA in urine with a long terminal urinary half-life of 25 hours. Thus, MA had been detected in urine 7 days after using a regimen of 10 mg four daily of MA (Oyler et al., 2002).

Table 2 A summary of the pharmacokinetic profile of MA (Cruickshank and Dyer, 2009)

	Dose	Bioavailability		T_{max} (minutes)	$T_{1/2}$ (hour)	Time to peak effect
Intravenous	30 mg	100%	108 ± 22 (64-164)	6 ± 11[b]	9.1 ± 0.8 (8–16)	<15 minutes
Smoking	30 mg	67%[d]; 90 ± 10 %[c]	47 ± 6	150 ± 30	12 ± 1 (8–17)	18 ± 2 minutes
Oral	30 mg[e]	67 ± 3%	94.1 (62–291)	216 (180–300)	9.1 (3–17)	180 minutes[a]
Intra-nasal	50 mg	79%	113 ± 8	169 ± 8	11± 1 hours[a]	15 minutes[a]

Cmax: peak plasma methamphetamine concentration; Tmax: time to reach peak plasma methamphetamine concentration; T1/2: methamphetamine plasma half-life. Data are presented as mean ± standard error and/or (range) where available. [a]Peak effect estimated from published plots of subjective effect versus time. [b]Geometric mean, determined by non-compartmental analysis; may be overestimated due to sampling interval. [c]Based on the inhaled dose, does not include drug residue remaining on the pipe [11]. [d]Data from Harris et al., 2003 [32]. [e]Administered dose was 30 mg/70 kg.

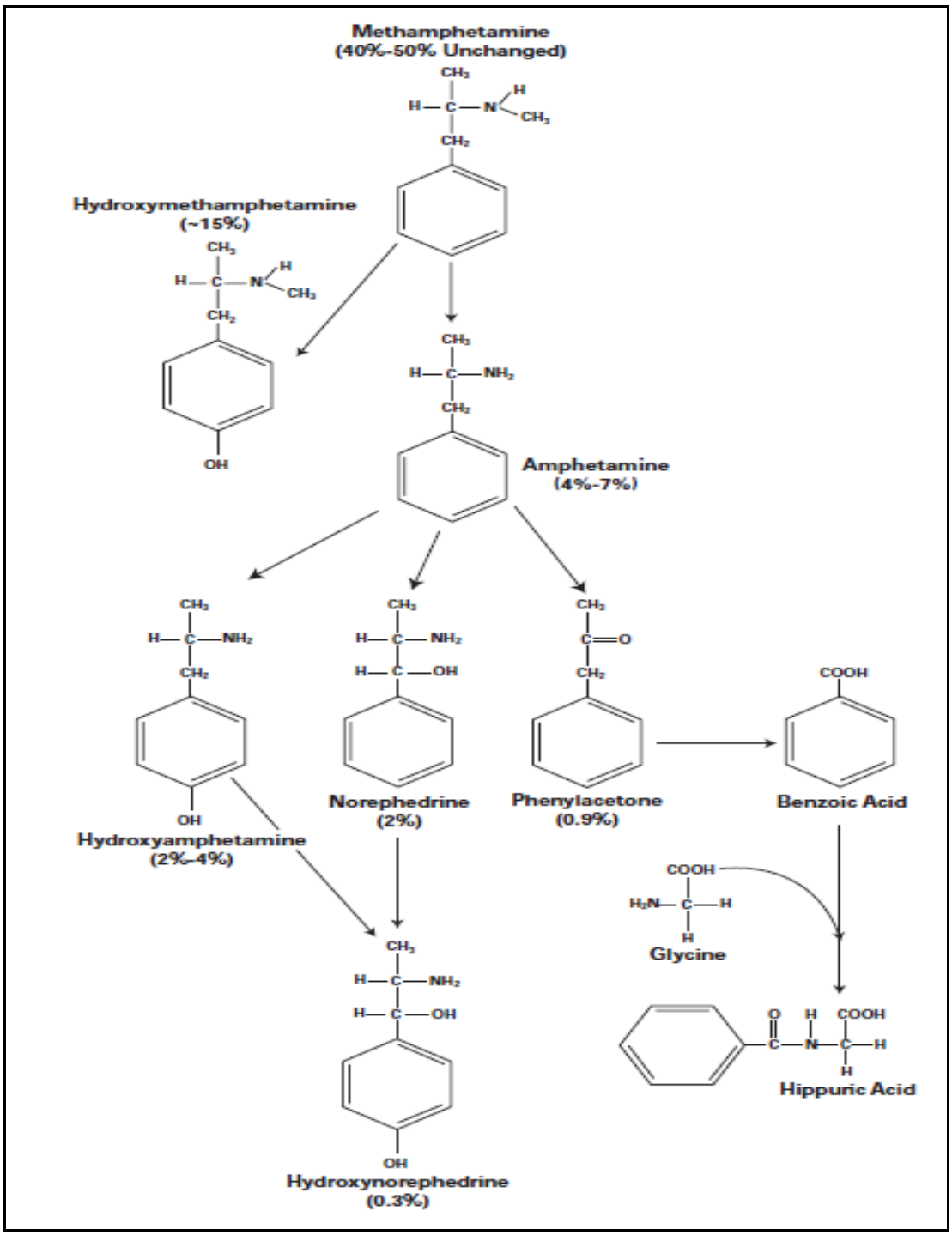


Figure 7 Metabolic pathways of methamphetamine (Moore, 1999)

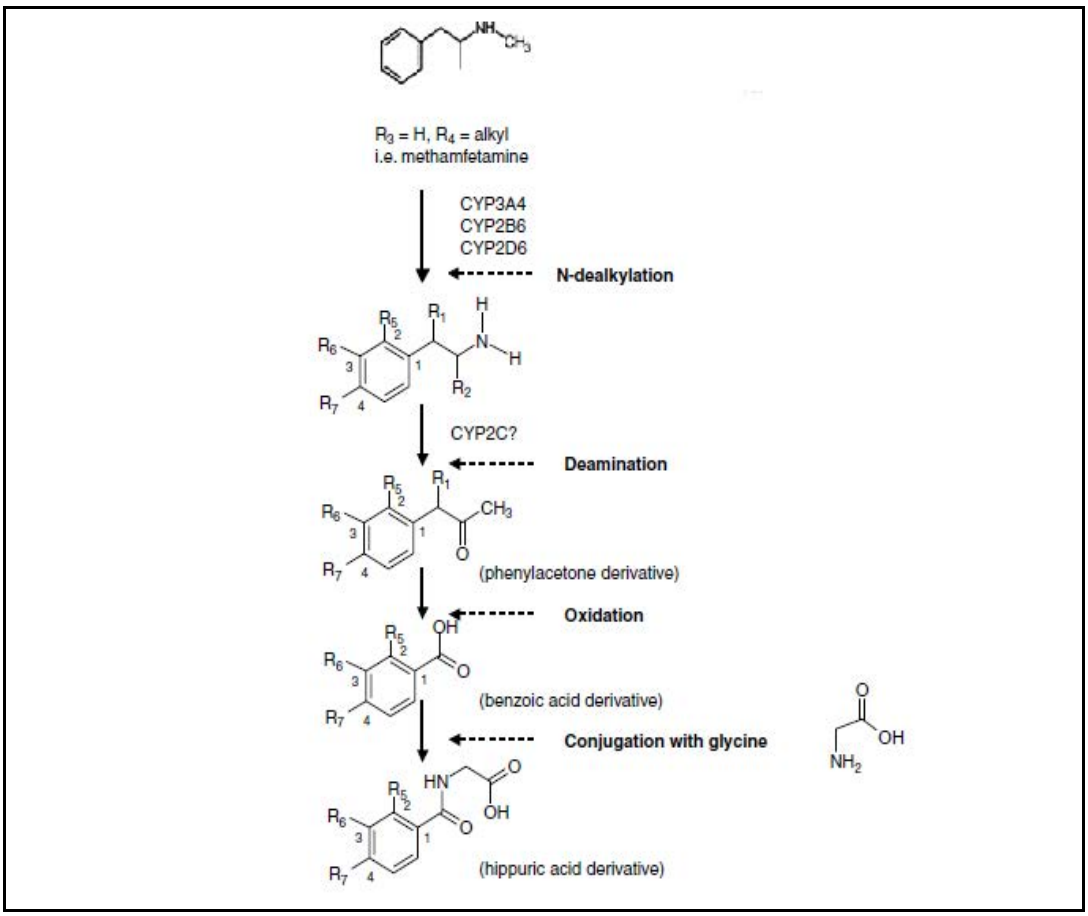


Figure 8 Metabolic reaction of methamphetamine resulting in amphetamine and the metabolites of amphetamine (Torre et al., 2004)

Determination of MA and AM in urine samples

MA has been used for many years. Although it is approved for medical purposes, its use is limited to a relatively small number of medical conditions. Large number of uses are for addictive drug. In Thailand, MA is classified as type I narcotic drug according to The Thai regulation. In general, determination of MA or other drugs of abuse in the body includes two processes. First, immunoassay or color test are usually used for urine screening in order to exclude the negative urine samples. Positive urine samples must be confirmed by a second confirmatory test. Gas chromatography-mass spectrometry is the most widely used method for confirmation (Cody, 1992; Kramer, 1998) since it provides high levels of specificity and sensitivity. In Thailand, urine MA or derivatives of AM concentration of $\geq$ 1000 ng/ml indicates the presence of these substances in the body. Thus, urine concentration of these substances of $\geq$ 1000 ng/ml is used as the cut-off value for positive interpretation of illicit abuser of MA or

derivatives of AM according to the regulation. In addition, MA and AM are metabolites of several componds such as amphetaminil, benzphetamine, clobenzorex, dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorex, mefenorex, mesocarb, methamphetamine, prenylamine, and selegiline which are therapeutically or illegally uses (Table 3) (Wang et al., 2005). These compounds are metabolically (bis) dealkylated to amphetamine or methamphetamine which can cause positive MA or AM results in urines resulting in the false interpretation as MA abusers.

However, metabolites of selegiline are primarily *l*-MA, *l*–AM and *N*-desmethylselegiline, not the *d*-MA and *d*-AM which are metabolites of the illicit MA. In routine analysis, currently used achiral identification techniques cannot differentiated stereoisomers. Thus, to determine the stereospecific metabolites, chiral derivatizing reagent such as (*S*)-(—)-*N*-(trifluoroacetyl)-propyl chloride (TPC), was added to perform derivatization before analyzed by chromatography (Wang, 2005; Tzing et al., 2007). Chiral column such as heptakis-β-cyclodextrin is also used to differentiate the stereoisomers (Hasegawa et al., 1999). Both methods can be used with GC-MS (Frank et al., 1978; Konig and Benecke, 1981; Liu et al., 1982; Hasegawa, 1999; Chang et al., 2001; Chiu et al., 2004; Tzing et al., 2006) and LC-MS (Wainer and Doyle, 1983; Armstrong, 1987; Karnes and Sarkar, 1987; Pirkle et al., 1987).

Several previous studies determined the stereoisomers of selegiline metabolites in urines and MA or AM metabolites in urine of MA users as following

1. Meeker and Reynolds. (1990)

This study analyzed the matabolites of selegiline in urine of a dead person (72 years old, male). He had a history of Parkinson's disease and treated with selegiline. Quantitation of MA and AM was performed by GC-MS and derivatized with pentafluoroproprionic anhydride (PFPA). They found that urine MA and AM concentrations were 2.38 and 0.72 μg/ml, respectively. Thus the AM/MA ratio was 0.30.

2. Kupiec et al. (1999)

This study reported the concentration of MA and AM in the urine samples collected from a dead body with aircraft accident. He previously received selegiline for Parkinson's disease. Analysis was performed by GC-MS with TPC as a derivatizing reagent. The concentrations of *l*-MA and *l*-

AM in the urine samples were 685 and 320 ng/ml, respectively. The urinary ratio of AM/MA was 0.46.

3. Fugita et al. (1999)

This study detected the stereoisomer of *l*-MA and *l*-AM in urine sample of a patient (44 years old, male) with selegiline overdosage (30 mg). Determination of the metabolite stereoisomers was performed by GC-MS using *l*-TPC as a chiral derivatizing reagent. The concentrations of *l*-MA and *l*-AM in urine sample were 0.62 and 0.25 μg/ml, respectively. Thus, the urinary ratio of AM/MA was 0.40.

4. Hasegawa et al. (1999)

This study was performed in 14 healthy volunteers. The subjects were given selegiline 2.5 mg, 5 mg and 10 mg once daily for 7 days. Urine samples were collected and analyzed for *l*-MA and *l*-AM using GC-MS with the chiral column coated with heptakis-β-cyclodextrin. Urine samples of 50 street illicit MA users were also collected and analyzed for *d*-MA and *d*-AM in the same manner as the patients. They found that the ratio of AM/MA gradually increased from 0.24 to 0.67 ($r = 0.857$) along with time after the selegiline administration similarly among different selegiline dosage regimens. In contrast, the urinary AM/MA was less than 0.24 in 75% of the fifty MA abusers.

5. Kim et al. (2000)

This study was performed in male healthy volunteers receiving selegiline and MA abusers. Determinations of *d*-MA or *l*-MA and *d*-AM or *l*-AM were performed using capillary electrophoresis with the chiral column using carboxy methylated-β-cyclodextrin. The results showed that the ratio of AM/MA of selegiline users was 0.33 which was significantly higher than the ratio of AM/MA of MA abusers which was 0.02.

Table 3 A summary of some common MA and AM generating drug modified from Wang et al. (2005)

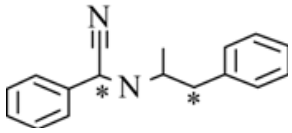
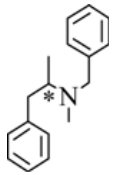
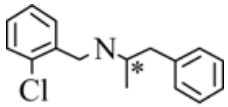
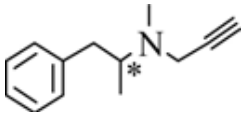
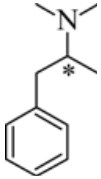
Brand name	IUPAC name	Chemical structure	Medical or illegal status	Important metabolites
Amphetaminil	α-[(1-Methyl-2-phenylethyl) amino]benzene cetonitrile		Psychotropic drug	AM
Benzphetamine	*N*, α-Dimethyl-*N*-(phenylmethyl)-benzeneethanamine		Treatment of obesity	AM; MA; 1-(4-Hydroxyphenyl)-2-(*N*-methyl-*N*benzylamino) propane
Clobenzorex	*N*-[(2-Chlorophenyl)methyl]-α-methylbenzeneethanamine		Treatment of obesity	AM; 4-Hydroxyamphetamine; 4-Hydroxyclobenzorex
Deprenyl (selegiline)	*N*, α-Dimethyl-*N*-2-propenyl-benzeneethanamine		Treatment of Parkinson's disease	MA; AM; Desmethyldeprenyl
Dimethylamphetamine	*N*,*N*-α-Trimethyl-benzeneethanamine		No recognized medical use; an illicit drug	MA; AM; Dimethylamphetamine- *N*-

Table 3 (Continued)

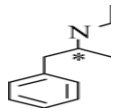
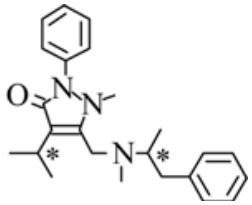
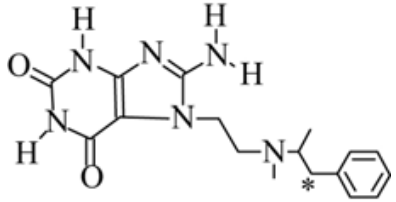
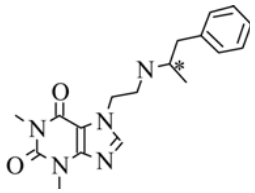
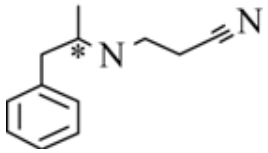
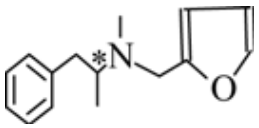
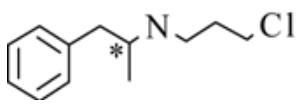
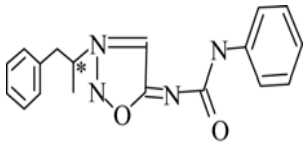
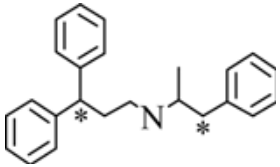
Brand name	IUPAC name	Chemical structure	Medical or illegal status	Important metabolites
Ethylamphetamine	*N*-Ethyl-α-methylbenzeneethanamine		Schedule I drug in USA; no recognized medical use	AM; 4-Hydroxyethylamphetamine
Famprofazone	4-Isopropyl-2-methyl-3-[*N*-methyl-*N*-(α-methyl-phenylethyl)-minomethyl]-1-phenyl-3-pyrazolin-5-one		Antipyretic & analgesic	MA; AM; 3-Hydroxymethylpyrazolone
Fencamine	*N*-Methyl-*N*-(1-methyl-2-phenylethyl)-*N*-3,7-dihydro-1,3,7- trimethyl-8-[[2-[methyl(1-methyl-2-phenylethyl)amino]ethyl]amino]-1H-purine-2,6-dione		Treatment of depression	MA; AM
Fenethylline	3,7-Dihydro-1,3-dimethyl-7-[2-[(1-methyl-2-phenylethyl) amino]ethyl]-1H-purine-2,6-dione		Schedule I drug in USA; treatment of narcolepsy and children with attention deficit disorder	AM; Theophylline; Hippuric acid

Table 3 (Continued)

Brand name	IUPAC name	Chemical structure	Medical or illegal status	Important metabolites
Fenproporex	3-[(1-Methyl-2-phenylethyl)amino]-propanenitrile		Treatment of obesity	AM
Furfenorex	*N*-Methyl-*N*-(1-methyl-2-phenylethyl)-2-furanmethanamine		Treatment of obesity	AM; MA; 1-Phenyl-2-(*N*methyl-*N*-γ-valerolactonylamino) propane
Mefenorex	*N*-(-3-Chloropropyl)-α-methyl-benzeneethanamine		Treatment of obesity	AM; 4-Hydroxymefenorex
Mesocarb	3-(1-Methyl-2-phenylethyl)-*N*-(phenylaminocarbonyl)-sydnoneimine		A stimulant; treatment of phantom pain syndrome	AM; Hydroxymesocarb; Dihydroxymesocarb
Prenylamine	*N*-(1-Methyl-2-phenylethyl)-γ-phenyl-benzenepropanamine		A coronary vasodilator; treatment of angina	AM; Norephedrine; Diphenylpropylamine

CHAPTER III

MATERIAL AND METHODS

Chemicals

1. Amphetamine (AM) hydrochloride, Lipomed (U.S.A.)
2. Diphenhydramine hydrochloride, Sigma Chemical Ltd. (U.S.A.)
3. Potassium hydroxide (KOH) , Sigma Chemical Ltd. (U.S.A.)
4. Methamphetamine (MA) hydrochloride, Lipomed (U.S.A.)
5. Ethanol, Sigma Chemical Ltd. (U.S.A.)
6. Sodium chloride (NaCl), Sigma Chemical Ltd. (U.S.A.)

Instruments

1. Auto pipettes 10, 100, 1000 µl, pipette tips
2. Gas chromatographic-mass spectrometry (GC-MS, QP-2010 plus, Shimadzu, Kyoto, Japan) with AOC-5000 Auto injector (Shimadzu, Kyoto, Japan)
3. Glass vial 20 ml, silicone septum cap and aluminum crimp seal
4. SPME fiber, Supelco (Bellefonte, PA, USA)

Patients

Male and female Thai patients were outpatients of Prasat Neurological Institute, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand. Patient's medical charts were reviewed and the patients were included into the study according to the following criteria:

Inclusion criteria

1. Thai patients of both sexes, male and female.
2. The patients received selegiline for at least 7 days before starting the sample collection. Selegiline dosage regimen was not changed during the study.

Exclusion criteria

1. Patients who had adverse drug reaction and/or other abnormal symptoms during the study.
2. Patients who were not compliance with using selegiline and urine sample collection.

Patients sample size was calculated as following:

$$N = \left[\frac{Z_\alpha + Z_\beta}{C} \right]^2 + 3$$

$$\text{Where } C = 0.5 \times \ln \left[\frac{1+ r}{1- r} \right]$$

N = number of patients

Z_α = Z – value at specific α error

Z_β = Z – value at specific β error

r = correlation coefficient from a previous study

The value of r = 0.857 used in this study was from the correlation coefficient between the ratio of AM to MA concentrations and times after selegiline administration reported by Hasegawa et al. (1999).

Assume that $\alpha = 0.05$, $\beta = 0.10$, $Z_\alpha = 1.645$, $Z_\beta = 1.282$, C = 1.281

$$\text{Therefore, } N = \left[\frac{Z_\alpha + Z_\beta}{C} \right]^2 + 3$$

$$= \left[\frac{1.645+1.282}{1.281} \right]^2 + 3$$

$$= (2.2849)^2 + 3$$

$$= 8.22$$

The lowest number of patients was 10. Urines were collected from each patient for five times points (2, 4, 6, 8 and 20 hours) after selegiline ingestion.

Ethical approval

The study protocol was approved by the ethical committee on the protection of rights of human subjects of the Prasat Neurological Institute (Approval # 0310 (12500) / 2.250, March 2, 2011).

MA abusers

MA abusers who had used MA were included into the study only the ones whose the information of the time of last use of MA and the time of urine sample collection could be recorded. Their urine samples were sent to the Institute of Forensic Medicine, Police General Hospital, Bangkok, Thailand for forensic analysis. The lowest number of abusers included into the study was 70.

Methods

1. Urine collection from patients

Fifteen Thai patients receiving selegiline were recruited into the study to assess the correlation between the ratio of AM to MA concentrations in urine and time after selegiline administration. Urine samples of each patient were collected at 2, 4, 6, 8, and 20 hours after selegiline administration. The ratio of AM/MA in urines of patients in this group was also used to compare with the ratio of AM/MA in urines of MA abusers.

Each recruited patient was followed up for 2 visits and the following activities were performed in the individual patient as following:

1.1 The first visit

1.1.1. At the first visit, patients' medical charts at the outpatient department of Prasat Neurological Institute were reviewed. Patients who had received selegiline for at least 7 day were selected.

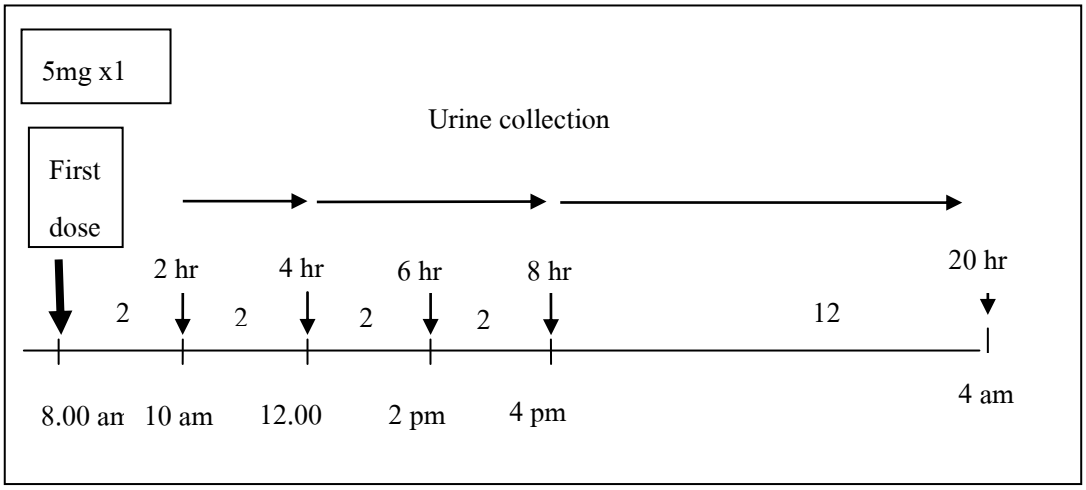
1.1.2. Detail of the study was explained to each patient according to the research subject information (Appendix 1B).

1.1.3. Patients who were interested to be included into the study were asked to sign the informed consents (Appendix 2B).

1.1.4. Urine samples were collected at 2, 4, 6, 8, and 20 hours after selegiline administration.

1.2 The second visit

Urine samples at the time points which could not be collected at the first visit were collected from the patients. Design of urine collection from patients was shown in Figure 9.



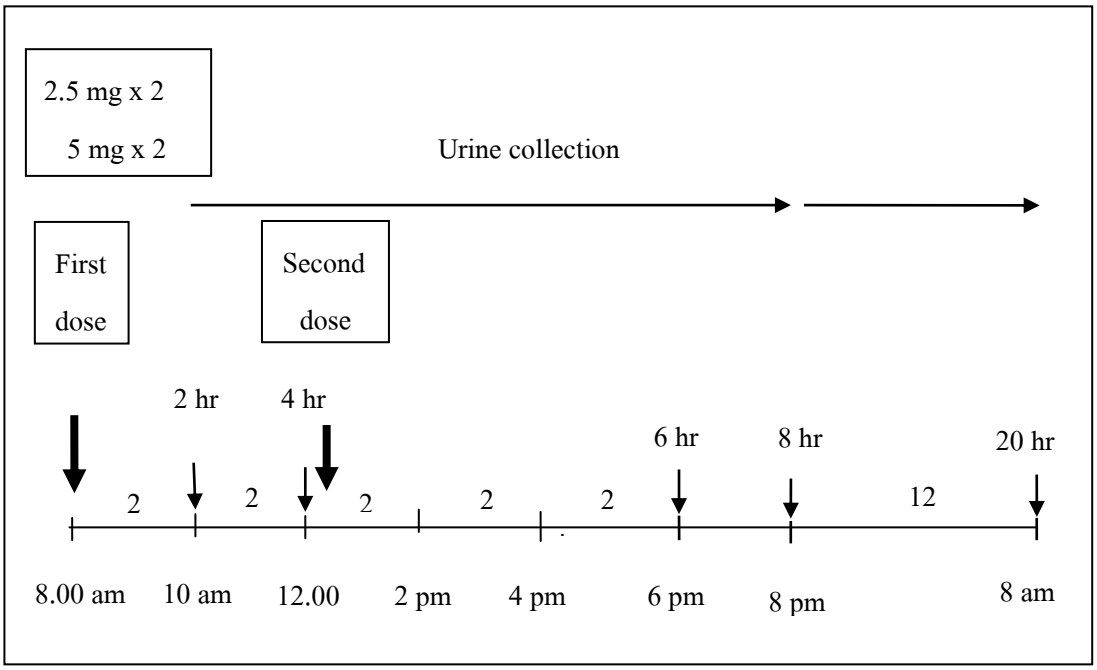


Figure 9 Urine collection from patients received selegiline at different dosage regimens

2. Urine collection from MA abusers

Urine samples were collected from the MA abusers whose the information of time of last MA use and the time of urine sample collection could be recorded. These informations of times were provided by the police officers who collected the urine samples and sent the samples to Institute of Forensic Medicine for analysis. Urine samples were collected from 97 MA abusers at either 2, 4, 6, 8 or 20 hours after the last use of MA.

3. Validation of the assay procedure for determining MA and AM concentrations in urine samples

3.1. Preparation of stock standard solutions

Stock solutions of MA and AM were prepared by dissolving 1 mg of MA or AM in 1 ml of ethanol to yied the concentrations of 1 mg/ml. Five hundred microlitres of each stock solution was added to a 25 ml volumetric flask and the volume was adjusted with distilled water to obtain the final stock standard solution of 20 µg/ml of MA or AM.

3.2. Validation of the assay procedure for determining MA and AM concentrations in urine samples

3.2.1 Linearity assay

Twenty five, 50, 75, 100, 125 and 150 microlitres of final stock standard solution (20 µg/ml) of MA or AM were added to 20 ml vials. Blank urine obtained from pooled urine samples of healthy volunteers was added into each vial so as to achieve the final volume of 1000 µl. Thus, the final concentrations of MA or AM in the vials were 500, 1000, 1500, 2000, 2500 and 3000 ng/ml, respectively. These solutions were analyzed by SPME-GC-MS according to the procedure subsequently described. Analysis of each concentration of MA or AM was performed in triplicate. Linear regression and coefficient of determination (R^2) between MA or AM concentrations and peak area ratio of standard solution to internal standard were analyzed.

3.2.2 Accuracy assay

Accuracy of the assay procedure was assessed by the percentage of recovery which was evaluated by comparing MA or AM concentrations between the measured concentration and the actual concentration of three concentrations of 500, 1500, and 2500 ng/ml of MA or AM. Three concentrations of MA or AM were analyzed by SPME-GC-MS, five times for each concentration. The percentage of recovery was calculated as following:

$$\% \text{ Recovery of MA} = \frac{\text{Measured MA concentration}}{\text{Actual MA concentration}} \times 100$$

or

$$\% \text{ Recovery of AM} = \frac{\text{Measured AM concentration}}{\text{Actual AM concentration}} \times 100$$

3.2.3 Precision assay

Precision of the assay was evaluated as within day and between day precision and assessed from the percentage of coefficient of variation (% CV) as following:

$$\% \text{ CV} = \frac{\text{Standard deviation (SD)}}{\text{Mean}} \times 100$$

Within day precision

MA or AM concentrations of 500, 1500 and 2500 ng/ml were analyzed by SPME-GC-MS five times for each concentration within 24 hours.

Between day precision

MA or AM concentrations of 500, 1500 and 2500 ng/ml were analyzed by SPME-GC-MS for 5 days. Each concentration was analyzed three times in each day of analysis.

4. Determination of MA or AM concentrations in urine using SPME-GC-MS

MA and AM concentrations in urine samples were determined using the method modified from the method of Myung et al. (1998). One millilitre of urine sample was placed in a 20 ml vial and 300 µl of a mixture (1:10 v/v) of diphenhydramine (4 mg/ml) and 200 mM KOH was added. After 3 g of sodium chloride was added, the vial was sealed with a silicone cap and an aluminum crimp seal. MA and AM concentrations in urine samples were analyzed by SPME-GC-MS.

The GC-MS was equipped with a 30 m x 0.25 mm (i.d.) column, Rtx-1MS (Restex, U.S.A). The column oven was set at 100 °C for 5 min and then programmed to increase from 100 to 150 °C at 15 °C/min for 1 min and finally increase to 250 °C for 3 min. The injection port and interface temperature were set at 240 °C and 220 °C, respectively. Splitless injection mode was

used. Helium with flow rate of 1.53 ml/min was used as the carrier gas. Quantification of sample was done by the selective ion monitoring (SIM) method and selected characteristic ions for AM and MA were *m/z* = 44 and 58, respectively.

SPME assembly with a replaceable extraction fiber, coated with 100 μm polydimethylsiloxane, was equipped with an AOC-5000 Auto injector. The samples were adsorption for 10 min and fiber were desorbed for 6 min.

5. Data analysis

Data were presented as mean ($\overline{X}$) ± standard deviation (SD) or standard error of the mean (SEM).The difference between the ratio of AM to MA concentrations in the urine samples of Thai patient receiving selegiline and those in the urine samples of MA abusers were analyzed by Mann-Whitney test. The correlations between MA or AM concentrations and times after selegiline administration or MA uses were assessed by Pearson's correlation test or Spearman correlation test. The correlations between MA or AM standard concentrations and peak area ratio of MA or AM to those of the internal standard were assessed by Pearson's correlation test. Statistic analysis was performed using SPSS version 16. The difference was considered to be statistically significant at $p < 0.05$.

CHAPTER IV

RESULTS

1. Validation of the assay procedure for determining of MA and AM concentrations

1.1 Linearity

Linearity was determined using 6 different concentrations (500, 1000, 1500, 2000, 2500 and 3,000 ng/ml) of standard MA or AM in blank pooled urine samples. Each standard MA or AM concentration was analyzed for 3 times. The linear regression equation and coefficients of determination (R^2) of the correlation between MA or AM concentrations and the peak area ratio of MA or AM to the internal standard were shown in Figure 10 for MA and those for AM were shown in Figure 11. Using this assay procedure, MA or AM concentrations and peak area ratio of MA or AM to internal standard were linearly correlated with $R^2 = 0.999$ ($P < 0.001$) for MA and and $R^2 = 0.999$ ($P < 0.001$) for AM.

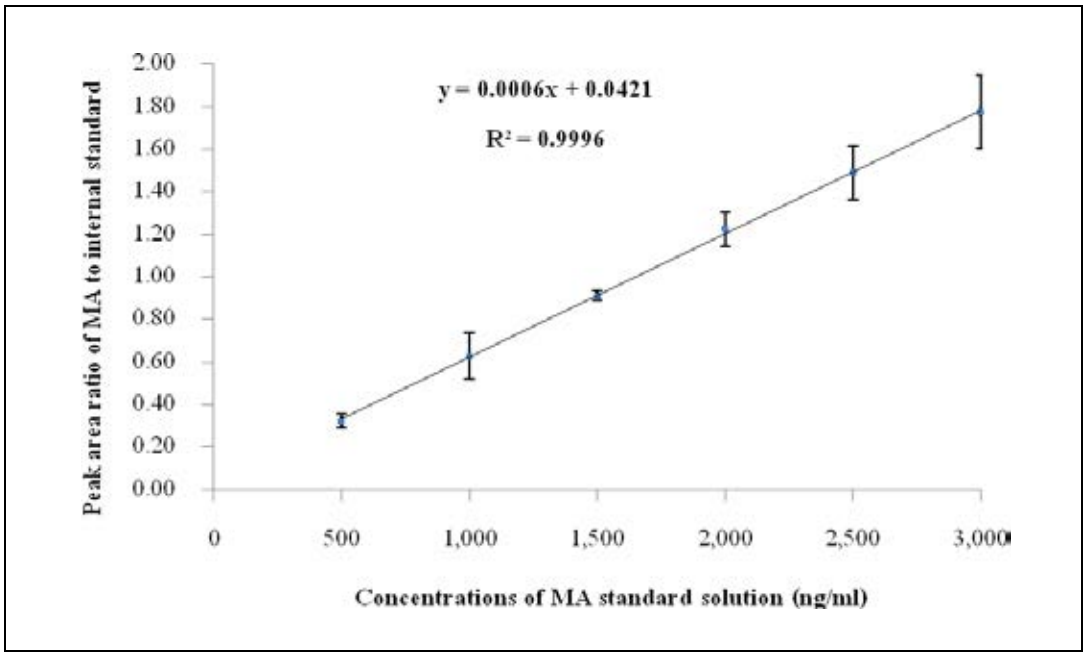


Figure 10 Correlation between MA standard concentrations and peak area ratio of MA to internal standard. The data shown were mean and SD of n = 3

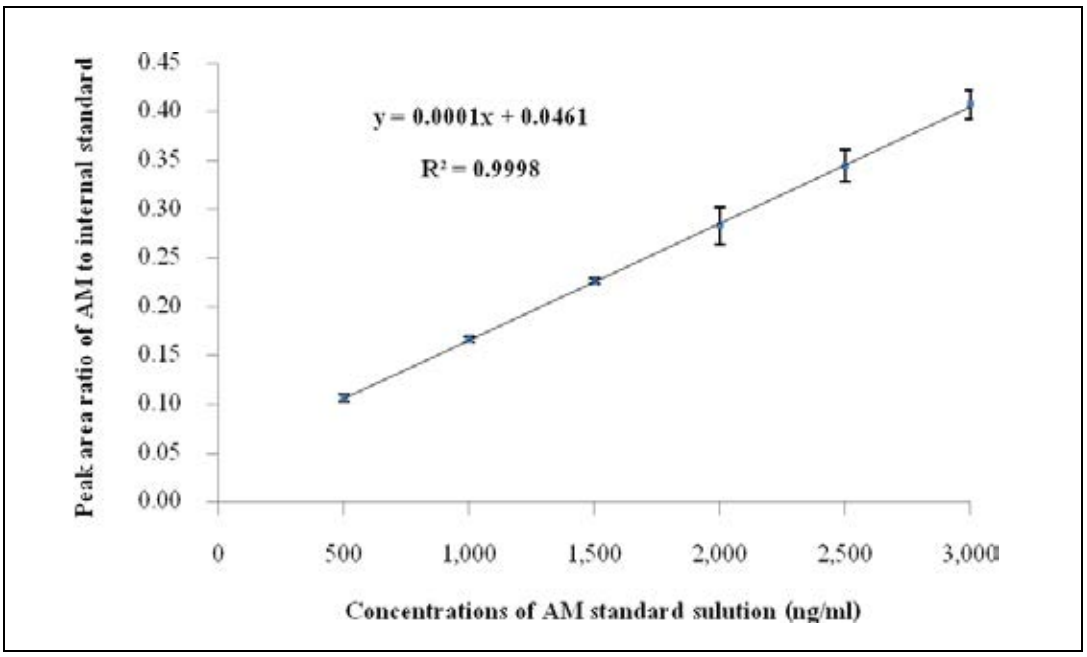


Figure 11 Correlation between AM standard concentrations and peak area ratio of AM to internal standard. The data shown were mean and SD of n = 3

1.2 Accuracy

Accuracy of the assay procedure was assessed by the percentage of recovery. Three MA or AM concentrations (500, 1500, and 2500 ng/ml) were analyzed for MA or AM concentrations using SPME-GC-MS five times for each concentration. The percentage recovery of MA were 101.11%, 99.64% and 100.53% for the MA concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 4). The percentage recovery of AM were 101.45%, 101.29%, 99.95% for the AM concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 5).

1.3 Precision

1.3.1 Within day precision of the assay procedure was performed with three concentrations (500, 1500 and 2500 ng/ml) of MA or AM within the same day for five times at each concentration. The precision was shown by the % CV of 4.54, 1.65, 1.68 for MA concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 6), while the % CV was 3.94, 1.32 and 1.49 for AM concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 7).

1.3.2 Between day precision of the assay procedure was performed with three concentrations (500, 1500 and 2500 ng/ml) of MA or AM. Each concentration of MA or AM was analyzed by SPME-GC-MS three times for each day and performed for 5 consecutive days. Percent coefficient of variation was shown to be 0.33, 0.97 and 0.58 for MA at concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 8) while the % CV was shown to be 1.99, 0.69 and 0.62 for AM at concentrations of 500, 1500 and 2500 ng/ml, respectively (Table 9).

Table 4 Accuracy of the assay procedure for determination of MA concentrations in urines

MA concentrations (ng/ml)		500	1500	2500
Measured MA concentrations	vial 1	504.65	1527.70	2524.48
	vial 2	488.85	1513.13	2547.81
	vial 3	484.71	1484.69	2556.01
	vial 4	506.61	1475.18	2477.45
	vial 5	542.83	1472.13	2460.94
Mean		505.53	1494.57	2513.34
% recovery		101.11	99.64	100.53
Mean of % recovery		100.43		

Table 5 Accuracy of the assay procedure for determination of AM concentrations in urines

AM concentrations (ng/ml)		500	1500	2500
Measured AM concentrations	vial 1	523.18	1531.07	2530.28
	vial 2	529.30	1534.03	2442.24
	vial 3	486.38	1484.78	2491.36
	vial 4	510.33	1519.96	2495.17
	vial 5	486.99	1526.79	2535.10
Mean		507.24	1519.33	2498.83
% recovery		101.45	101.29	99.95
Mean of % recovery		100.90		

Table 6 Within day precision of the assay procedure for determination of MA concentrations in urines

MA concentrations (ng/ml)		500	1500	2500
Measured MA concentrations	vial 1	504.65	1527.70	2524.48
	vial 2	488.85	1513.13	2547.81
	vial 3	484.71	1484.69	2556.01
	vial 4	506.61	1475.18	2477.45
	vial 5	542.83	1472.13	2460.94
Mean		505.53	1494.57	2513.34
SD		22.94	24.59	42.33
% Coefficient of variation (% CV)		4.54	1.65	1.68

Table 7 Within day precision of the assay procedure for determination of AM concentrations in urines

AM concentrations (ng/ml)		500	1500	2500
Measured AM concentrations	vial 1	523.18	1531.07	2530.28
	vial 2	529.30	1534.03	2442.24
	vial 3	486.38	1484.78	2491.36
	vial 4	510.33	1519.96	2495.17
	vial 5	486.99	1526.79	2535.10
Mean		507.24	1519.33	2498.83
SD		19.97	20.02	37.34
% Coefficient of variation (% CV)		3.94	1.32	1.49

Table 8 Between day precision of the assay procedure for determination of MA concentrations in urines

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured MA concentration	vial 1	504.65	508.02	513.36	516.66	492.85
(500 ng/ml)	vial 2	488.85	493.98	514.56	511.74	509.22
	vial 3	484.71	494.13	503.54	498.98	494.84
Mean		492.74	498.71	510.49	509.13	498.97
Mean				502.01		
SD				1.65		
% CV				0.33		

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured MA concentration	vial 1	1527.71	1524.87	1510.09	1514.03	1559.23
(1500 ng/ml)	vial 2	1513.13	1474.10	1497.86	1497.61	1469.60
	vial 3	1484.69	1490.55	1477.64	1495.84	1483.03
Mean		1508.51	1496.51	1495.19	1502.49	1503.95
Mean				1501.33		
SD				14.60		
% CV				0.97		

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured MA concentration	vial 1	2524.48	2554.32	2557.09	2533.77	2551.96
(2500 ng/ml)	vial 2	2547.81	2522.78	2485.34	2509.50	2549.00
	vial 3	2556.01	2488.75	2578.56	2516.35	2491.71
Mean		2542.77	2521.95	2540.33	2519.87	2530.89
Mean				2531.16		
SD				14.69		
% CV				0.58		

Table 9 Between day precision of the assay procedure for determination of AM concentrations in urines

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured AM	vial 1	523.18	535.61	482.38	477.26	480.89
concentration	vial 2	529.30	479.83	483.06	523.89	483.24
(500 ng/ml)	vial 3	486.38	503.33	489.57	528.92	518.52
Mean		512.95	506.25	485.00	510.02	494.22
Mean				501.69		
SD				10.00		
% CV				1.99		

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured AM	vial 1	1531.07	1544.74	1485.19	1514.03	1496.88
concentration	vial 2	1534.03	1523.07	1546.54	1497.61	1484.33
(1500 ng/ml)	vial 3	1484.78	1496.43	1549.29	1495.84	1513.04
Mean		1516.63	1521.41	1527.01	1502.49	1498.08
Mean				1513.12		
SD				10.48		
% CV				0.69		

Day of the assay		Day 1	Day 2	Day 3	Day 4	Day 5
Measured AM	vial 1	2530.28	2554.32	2520.21	2521.77	2513.50
concentration	vial 2	2442.24	2522.78	2514.49	2512.39	2535.04
(2500 ng/ml)	vial 3	2491.36	2488.75	2523.13	2482.95	2507.27
Mean		2487.96	2521.95	2519.28	2505.70	2518.60
Mean				2510.70		
SD				15.54		
% CV				0.62		

2. MA and AM concentrations and the AM/MA concentration ratio in urine samples of patients receiving selegiline

Demographic data of patients were shown in Table 10. It was shown that 11 patients were male and 4 patients were female. They are mostly old with the mean ± SD of their ages of 63.53 ± 9.21 years old (range 45-76 years old). Three different dosage regimens of selegiline were prescribed to these patient: 2.5 mg x 2 (4 patients), 5 mg x 1 (4 patients) and 5 mg x 2 (7 patients).

Concentrations of MA and AM in urine samples of patients collected at various times after selegiline administration and the corresponding AM/MA ratio were shown in Table 11. It was shown that MA and AM detected in urine samples of patients who were prescribed selegiline at therapeutic doses could be interpreted as MA or AM abusers. This was because high incidence of MA and AM was detected in urine samples of these patients at the concentration of > 1,000 ng/ml which is the cut-off value limited in the law. Percentages of false positive interpretation as MA users in these patients were shown to be 93.33%, 93.33%, 100%, 93.33%, 86.66% for the urines collected at 2, 4, 6, 8, and 20 hours after selegiline administration, respectively while all urine samples collected of all time points after selegiline administration showed AM concentrations of > 1,000 ng/ml. Thus, all urine samples (100%) showed false positive interpretation as AM users in these patients (Table 11).

Mean ± SEM of the ratio of AM/MA concentration in urine samples of the patients, collected at 2, 4, 6, 8 and 20 hours after selegiline administration were 0.92 ± 0.10, 0.80 ± 0.08, 0.74 ± 0.07, 0.91 ± 0.10, 0.98 ± 0.14, respectively (Table 11).

Table 10 Demographic data of patients (n = 15)

Patient No.	Age (years)	Sex	Dosage regimen of selegiline (mg/day)
1	68	male	2.5 mg x 2
2	76	male	2.5 mg x 2
3	58	female	2.5 mg x 2
4	64	male	2.5 mg x 2
5	70	female	5 mg x 1
6	76	male	5 mg x 1
7	45	female	5 mg x 1
8	71	male	5 mg x 1
9	59	male	5 mg x 2
10	68	male	5 mg x 2
11	55	male	5 mg x 2
12	52	male	5 mg x 2
13	68	male	5 mg x 2
14	54	male	5 mg x 2
15	69	female	5 mg x 2
$\overline{X}$ ± SD	63.53 ± 9.21		
range	45-76		

Table 11 Concentrations of MA and AM in urines of patients collected at various times after selegiline administration and the corresponding AM/MA ratio

Pateint No.	Concentrations of MA and AM (ng/ml) in urines of patients collected at various times after selegiline administration and the corresponding AM/MA ratio														
	2 hr			4 hr			6 hr			8 hr			20 hr		
	MA	AM	AM/MA	MA	AM	AM/MA	MA	AM	AM/MA	MA	AM	AM/MA	MA	AM	AM/MA
1	1136.58	1115.13	0.98	1304.28	1128.50	0.87	1579.99	1151.96	0.73	2169.66	1292.62	0.60	1515.85	1092.99	0.72
2	1247.64	1147.20	0.92	1027.37	1075.75	1.05	2625.15	1422.61	0.54	1533.20	1476.31	0.96	1504.93	1854.50	1.23
3	1090.38	1043.81	0.96	1808.98	1312.66	0.73	2847.72	1495.87	0.53	3342.88	1591.73	0.48	1598.41	1181.88	0.74
4	1043.63	1043.38	1.00	1855.29	1227.14	0.66	1133.26	1051.29	0.93	1323.20	1164.52	0.88	819.10	1025.30	1.25
5	1724.74	2541.82	1.47	2028.32	3100.77	1.53	1537.83	2293.72	1.49	2019.52	4008.87	1.99	1505.45	3830.87	2.54
6	1965.68	1557.65	0.79	2750.03	2057.79	0.75	1415.63	1455.80	1.03	1410.72	1367.06	0.97	1295.97	1382.79	1.07
7	2284.85	1400.24	0.61	2334.67	1589.39	0.68	2458.09	1935.02	0.79	1272.62	1187.66	0.93	866.77	1133.77	1.31
8	1345.78	1306.38	0.97	2102.73	1452.81	0.69	1701.31	1379.81	0.81	1319.22	1314.73	1.00	1655.86	1194.64	0.72
9	2348.58	1416.60	0.60	2011.36	1346.94	0.67	3163.46	1622.89	0.51	2749.60	1508.54	0.55	2716.62	1582.49	0.58
10	1153.97	2201.33	1.91	5119.15	3397.84	0.66	3074.41	2132.09	0.69	2511.50	2123.17	0.85	755.26	1149.64	1.52
11	3144.91	1153.05	0.37	2073.11	1036.35	0.50	3842.78	1328.87	0.35	726.98	1048.30	1.44	2143.04	1158.84	0.54
12	1882.38	1184.97	0.63	1430.47	1043.60	0.73	2014.99	1113.77	0.55	1218.74	1037.88	0.85	1662.07	1178.46	0.71
13	903.84	1034.15	1.14	1807.55	1060.88	0.59	1627.08	1047.02	0.64	1439.07	1029.96	0.72	1470.90	1060.23	0.72
14	2222.66	2466.33	1.11	993.58	1424.12	1.43	1432.11	1378.73	0.96	1462.03	1364.30	0.93	1935.08	1275.31	0.66
15	6171.15	2485.90	0.40	10582.11	4214.24	0.40	5854.35	3010.64	0.51	6809.67	3081.68	0.45	13509.34	5076.20	0.38
False positive (%) (>1,000 ng/ml)	93.33	100		93.33	100		100	100		93.33	100		86.66	100	
Mean			0.92			0.80			0.74			0.91			0.98
SEM			0.10			0.08			0.07			0.10			0.14

3. Relationship between the ratio of AM/MA concentrations and time after selegiline administration

Result from this study showed that the ratio of AM/MA concentrations in urines of patients receiving selegiline at different dosage regimens and the times (2, 4, 6, 8 and 20 hours) after selegiline administration was not linearly correlated (r = 0.300, p = 0.624) (Figure 12)

However, if the data were grouped according to the dosage regimen and the correlations between the AM/MA ratio and the times after selegiline administration were reassessed. It was shown that when selegiline was administered twice daily either 2.5 mg twice daily (Figure 13) or 5 mg twice daily (Figure 14), the ratio of AM/MA and the times after selegiline administration were not linearly correlated (r = 0.100, p = 0.873 for 2.5 mg twice daily regimen in Figure 13 and r = - 0.200, p = 0.747 for 5 mg twice daily regimen in Figure 14). In contrast, the ratio of AM/MA and the times after selegiline administration was linearly correlated (r = 0.926, p = 0.024) when selegiline was administered 5 mg once daily (Figure 15).

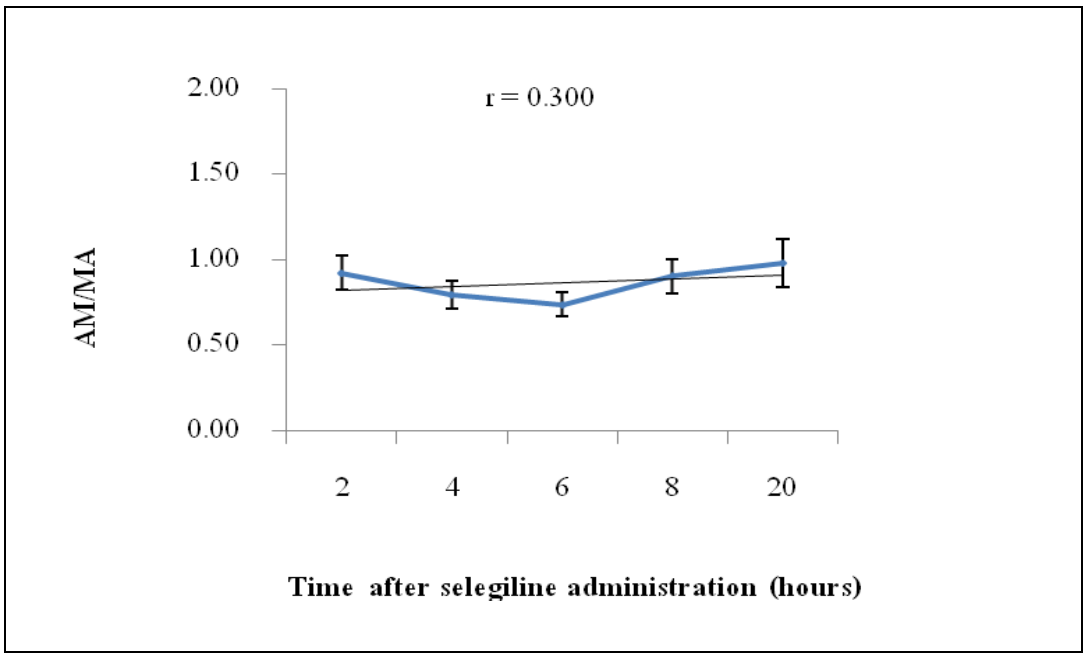


Figure 12 The correlation between ratio of AM/MA concentrations in urines of patients receiving selegiline at different dosage regimens and the times (2, 4, 6, 8 and 20 hours) after selegiline administration. The correlation was assessed by Spearman correlation test using SPSS version 16.

The data shown were mean ± SEM of n = 15.

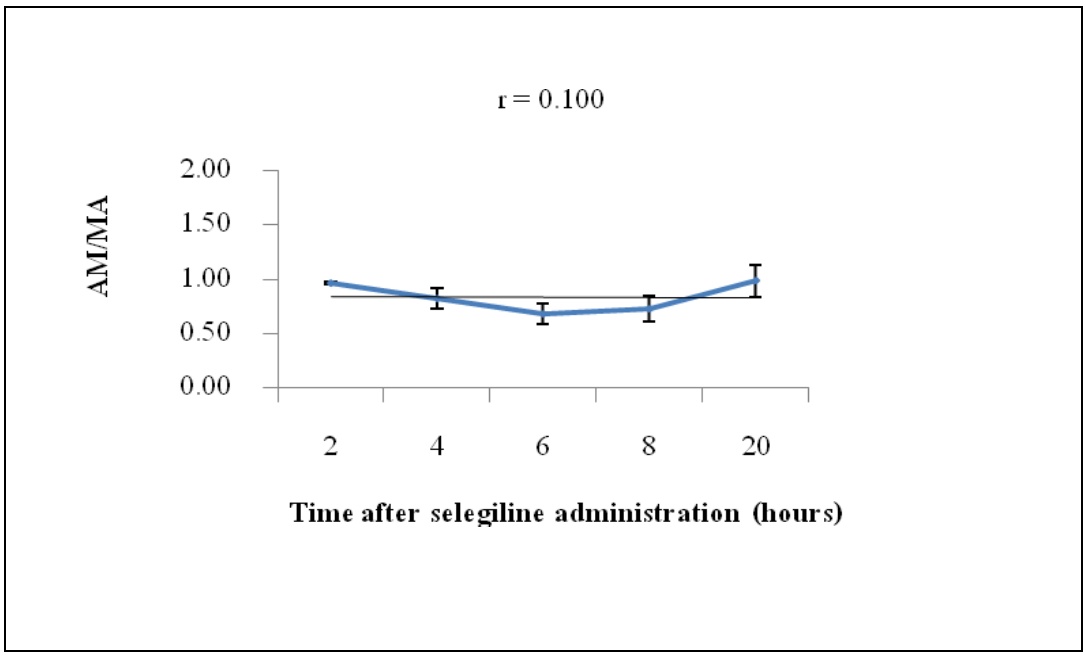


Figure 13 The correlation between ratio of AM/MA concentrations in urines of patients receiving selegiline 2.5 mg twice daily and the times (2, 4, 6, 8 and 20 hours) after selegiline administration. The correlation was assessed by Spearman correlation test using SPSS version 16.

The data shown were mean ± SEM of n = 4.

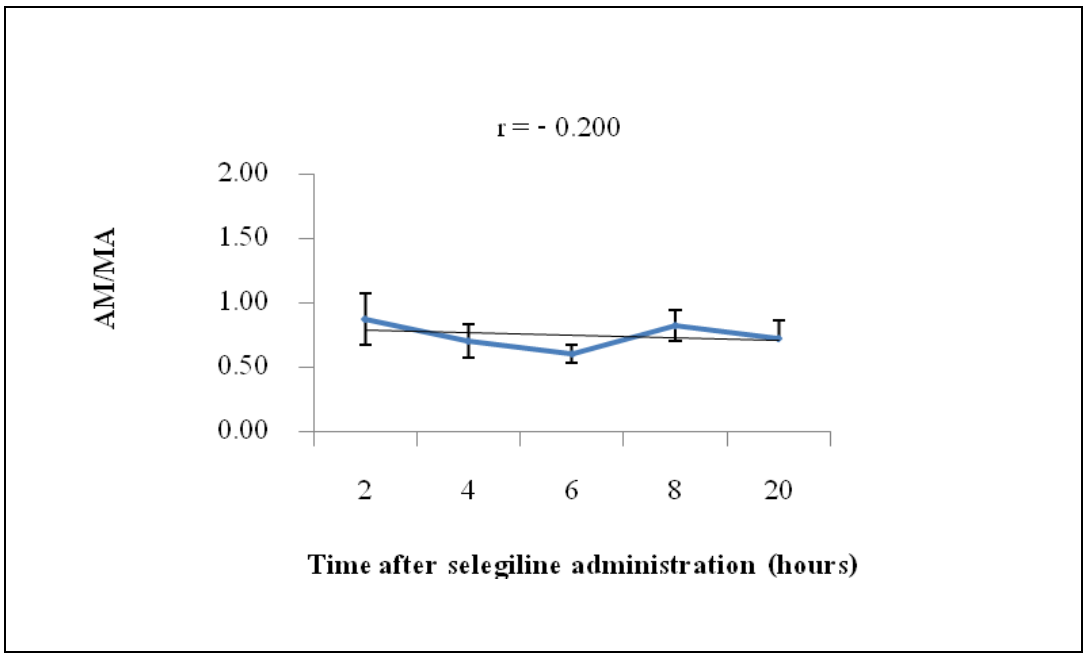


Figure 14 The correlation between ratio of AM/MA concentrations in urines of patients receiving selegiline 5 mg twice daily and the times (2, 4, 6, 8 and 20 hours) after selegiline administration. The correlation was assessed by Spearman correlation test using SPSS version 16.

The data shown were mean ± SEM of n = 7.

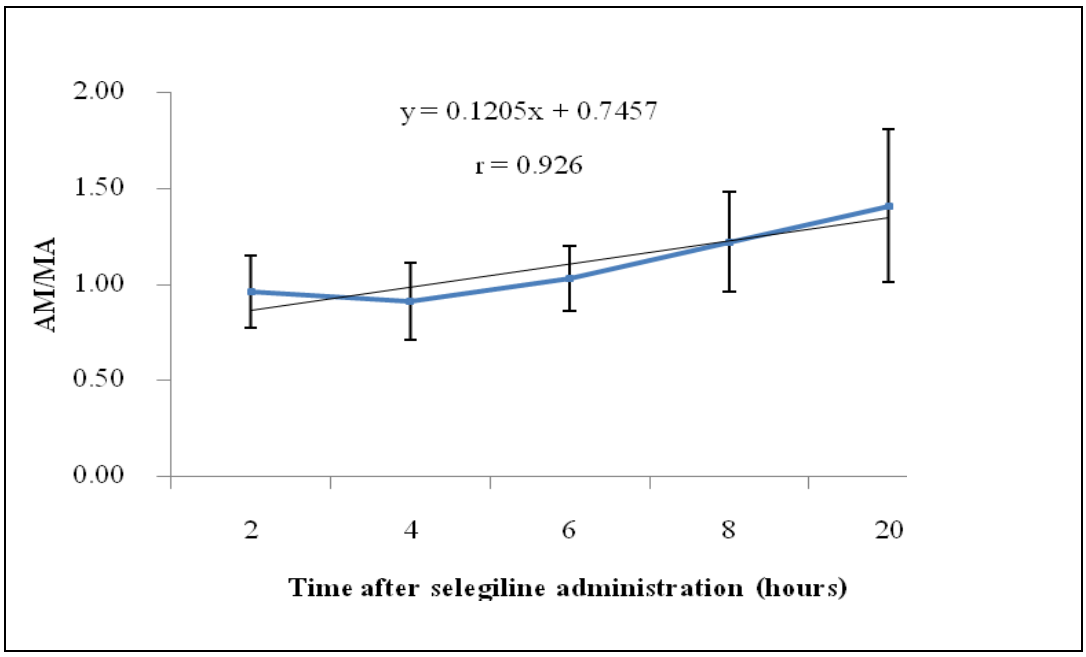


Figure 15 The correlation between ratio of AM/MA concentrations in urines of patients receiving selegiline 5 mg once daily and the times (2, 4, 6, 8 and 20 hours) after selegiline administration. The correlation was assessed by Pearson's correlation test using SPSS version 16.

The data shown were mean ± SEM of n = 4.

4. **The concentrations of MA and AM in urines of MA abusers collected at various times after MA uses**

Urine samples were collected from 97 abusers at various times (2, 4, 6, 8 or 20 hours) after MA uses. Among the MA abusers included into the study, 89 (91.75%) were male and 8 (8.24%) were female. Mean ± SEM of their ages were 28.46 ± 0.72 years (range of 16-48 years). Concentrations of MA and AM in urines of MA abusers collected at various times (2, 4, 6, 8 or 20 hours) after MA uses were shown in Table 12-16. It was shown that MA concentrations in urine samples of all MA abusers were more than 1000 ng/ml, thus, false negative interpretation of MA use were not shown in all MA abusers. Only small numbers of MA abusers had AM concentrations in their urines less than 1000 ng/ml. However, MA concentrations in their corresponding urine samples were more than 1000 ng/ml. There was no significant correlation between the ratio of AM/MA concentrations in urines of MA abusers and times after MA uses (Figure 16).

Significant differences were shown when the ratios of AM/MA concentrations in urine of MA abusers were compared to the ratios of AM/MA concentrations in urines of patients receiving selegiline at the same corresponding time point (2, 4, 6, 8 or 20 hours) after MA use (for MA abusers) or selegiline administration (for patients receiving selegiline) (Figure 17, Table 17). It was shown that the ratios of AM/MA concentrations in urines of patients were significantly higher than those of the MA abusers at every time point after selegiline administration or MA use. Among five time points of urine collection, AM/MA ratio of patients was lowest (0.74 ± 0.07) while the AM/MA ratio of MA abusers was highest (0.41 ± 0.05) at 6 hours after exposure to the compounds. To find the most reliable cut-off AM/MA ratio for differentiating patients receiving selegiline from MA abusers, the AM/MA ratios between 0.40 to 0.75 were tested using the AM/MA ratio data of 15 patients (15 patients x 5 time points of urine collection) and 97 MA abusers. Using the AM/MA ratio of 0.40 as the cut-off value, 72 patients were predicted as patients while 3 patients were predicted as MA abusers. For MA abusers, 74 MA abusers were predicted as MA abusers while 23 MA abusers were predicted as patients. Thus, using the AM/MA ratio of 0.4 as the cut-off value, accuracy of prediction was 84.88%. Using the cut-off value of 0.4 provided the highest probability that the patients receiving selegiline were predicted

as patients and provided the lowest probability of patients to be predicted as abusers (Table 18.1-18.16, Table 19).

Table 12 Concentrations of MA and AM in urines of MA abusers collected at 2 hours after MA use (n = 17)

MA Abuser (No.)	Age (years)	Sex	Concentration (ng/ml)		
			MA	AM	AM/MA
1	26	male	13411.01	2825.34	0.21
2	33	male	11162.74	2412.87	0.22
3	29	male	12655.38	1427.46	0.11
4	30	male	23237.19	1116.88	0.05
5	27	male	51362.22	9719.84	0.19
6	16	female	27959.92	1190.13	0.04
7	35	male	39330.07	5041.95	0.13
8	22	male	64298.92	8618.30	0.13
9	30	male	39628.41	10148.08	0.26
10	27	female	6728.32	1081.21	0.16
11	22	male	21037.80	2656.50	0.13
12	42	female	3907.18	1387.52	0.36
13	36	male	27614.54	2335.84	0.08
14	30	male	1781.55	987.78	0.55
15	31	male	2588.68	1270.17	0.49
16	38	male	9496.08	1464.23	0.15
17	36	male	2395.64	1188.76	0.50
Mean					0.22
SEM					0.04

Table 13 Concentrations of MA and AM in urines of MA abusers collected at 4 hours after MA use (n = 16)

MA Abuser (No.)	Age (years)	Sex	Concentration (ng/ml)		
			MA	AM	AM/MA
1	44	male	13145.83	1285.40	0.10
2	37	male	10835.93	1600.55	0.15
3	20	male	1081.41	990.26	0.92
4	17	male	6249.21	1375.66	0.22
5	27	female	19811.06	5891.30	0.30
6	30	male	30372.20	5132.04	0.17
7	27	male	17530.81	2300.19	0.13
8	27	male	4479.52	985.27	0.22
9	23	male	19372.35	1337.81	0.07
10	33	male	4206.99	1354.57	0.32
11	29	male	9398.00	1424.02	0.15
12	19	male	1895.56	1078.46	0.57
13	17	male	56558.51	2921.89	0.05
14	24	male	3154.24	1188.40	0.38
15	31	male	8817.58	1817.31	0.21
16	18	male	2714.08	1145.28	0.42
Mean					0.27
SEM					0.06

Table 14 Concentrations of MA and AM in urines of MA abusers collected at 6 hours after MA use (n = 21)

MA Abuser (No.)	Age (years)	Sex	Concentration (ng/ml)		
			MA	AM	AM/MA
1	17	male	20466.28	6312.62	0.31
2	23	male	24326.04	3964.43	0.16
3	25	male	2134.49	1519.86	0.71
4	19	male	2752.61	1663.53	0.60
5	33	male	1648.00	1347.44	0.82
6	28	male	1355.02	1123.42	0.83
7	20	male	8075.94	2124.11	0.26
8	36	male	13468.07	2460.29	0.18
9	36	male	8554.61	1627.70	0.19
10	32	male	1485.78	992.50	0.67
11	26	male	8081.59	7284.37	0.90
12	36	male	5623.57	1014.28	0.18
13	21	male	22394.28	6475.56	0.29
14	23	female	4751.85	1566.37	0.33
15	25	male	13399.84	1444.42	0.11
16	28	male	65637.52	7016.37	0.11
17	26	female	2925.62	1114.09	0.38
18	18	male	8947.10	3122.83	0.35
19	25	male	2022.01	1040.78	0.51
20	32	male	3326.21	1114.41	0.34
21	30	male	3816.97	1148.23	0.30
Mean					0.41
SEM					0.05

Table 15 Concentrations of MA and AM in urines of MA abusers collected at 8 hours after MA use (n = 20)

MA Abuser (No.)	Age (years)	Sex	Concentration (ng/ml)		
			MA	AM	AM/MA
1	22	male	42134.30	2574.30	0.06
2	41	male	3891.02	1262.41	0.32
3	35	male	10088.20	2409.67	0.24
4	30	male	9643.80	1342.87	0.14
5	21	male	1488.66	997.30	0.67
6	26	male	2906.52	1244.01	0.43
7	19	male	9492.86	4102.77	0.43
8	32	female	11453.69	6945.24	0.61
9	35	male	69985.62	8635.58	0.12
10	31	male	25423.02	4294.82	0.17
11	39	male	1102.05	1018.19	0.92
12	28	male	1275.20	1036.17	0.81
13	19	male	15254.34	1204.97	0.08
14	27	male	16843.55	985.56	0.06
15	27	male	40841.32	1696.64	0.04
16	31	male	18730.06	1980.41	0.11
17	19	male	3070.03	1076.10	0.35
18	30	male	4164.23	1092.74	0.26
19	38	female	14082.21	2003.55	0.14
20	30	male	1493.05	1203.27	0.81
Mean					0.34
SEM					0.06

Table 16 Concentrations of MA and AM in urines of MA abusers collected at 20 hours after MA use (n = 23)

MA Abuser (No.)	Age (years)	Sex	Concentration (ng/ml)		
			MA	AM	AM/MA
1	31	male	18457.23	1933.12	0.10
2	26	male	19145.69	1612.62	0.08
3	18	male	22005.81	2627.92	0.12
4	25	male	1415.46	1211.71	0.86
5	27	male	7666.65	2782.27	0.36
6	26	male	1473.10	1026.05	0.70
7	32	male	3739.18	1683.52	0.45
8	43	male	35829.93	5260.40	0.15
9	23	male	23613.88	3965.82	0.17
10	31	male	6489.30	985.25	0.15
11	20	male	21672.38	2607.53	0.12
12	40	male	9247.05	1770.18	0.19
13	18	male	16716.69	2602.40	0.16
14	48	male	17662.31	1352.35	0.08
15	23	male	4610.29	1125.51	0.24
16	26	male	9320.34	1398.84	0.15
17	29	male	72119.68	4524.48	0.06
18	35	male	31687.88	5221.54	0.16
19	23	male	7408.89	2833.23	0.38
20	36	male	30161.83	2094.56	0.07
21	48	male	6071.53	1534.01	0.25
22	33	male	9691.09	1657.13	0.17
23	28	male	1762.75	1051.13	0.60
Mean					0.25
SEM					0.04

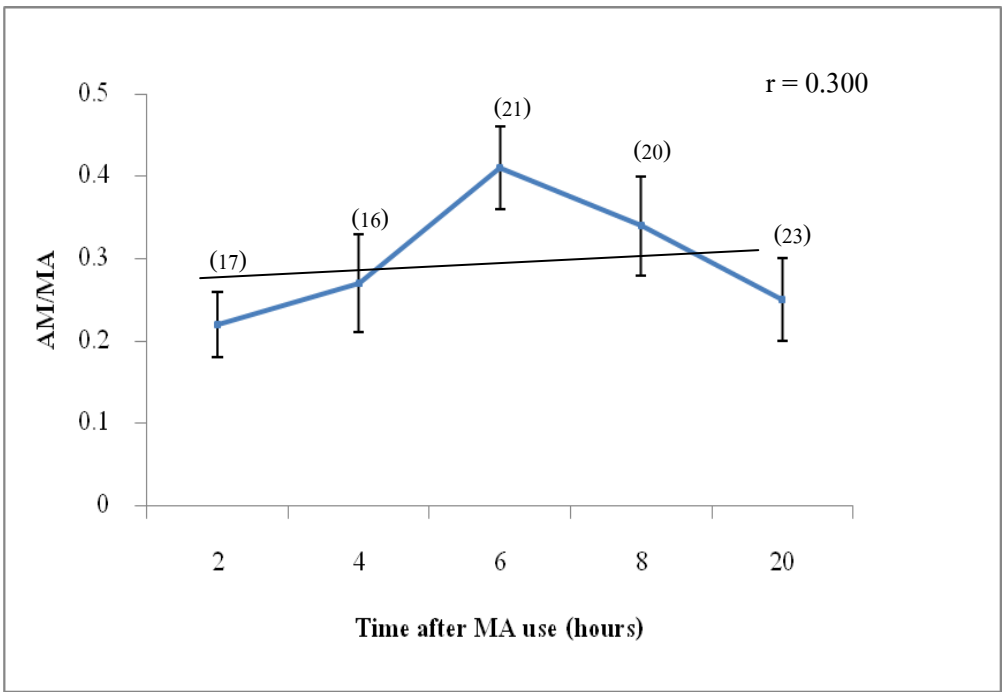


Figure 16 The correlation between ratio of AM/MA concentrations in urines of MA abusers and the times (2, 4, 6, 8 or 20 hours) after MA use.

The data shown were mean ± SEM with the sample size (n) shown in parentheses.

The correlation was assessed by Spearman correlation test using SPSS version 16.

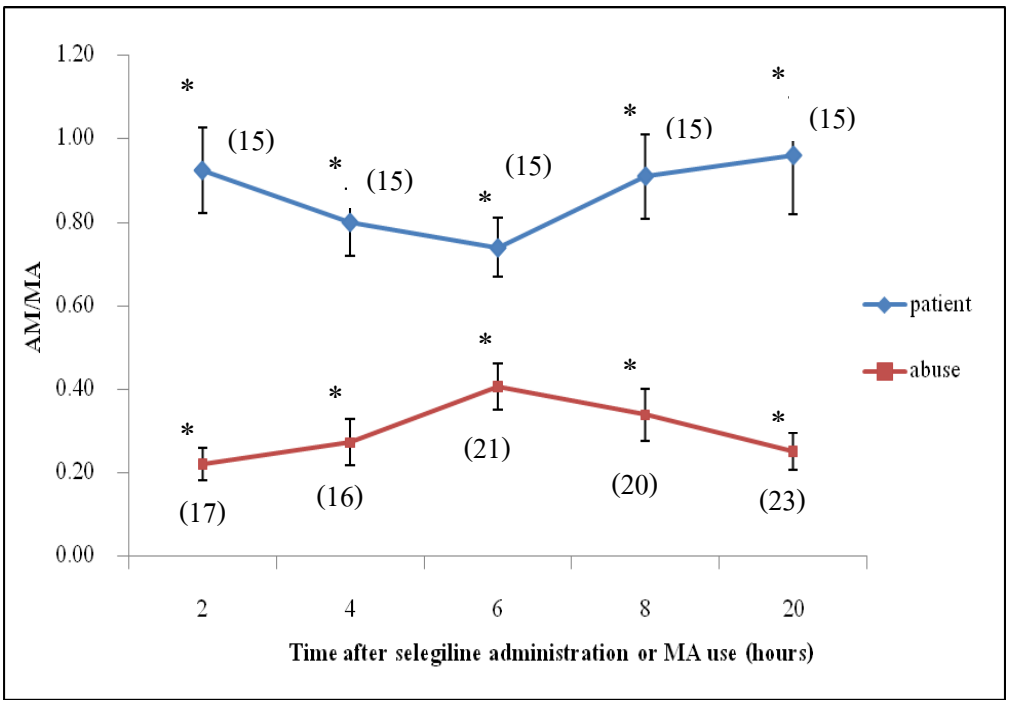


Figure 17 Comparison of AM/MA ratio between MA abusers and patients receiving selegiline at 2, 4, 6, 8 and 20 hours after MA use or selegiline administration.

The data shown were mean ± SEM with the sample size (n) shown in parentheses.

* $p < 0.01$; MA abusers vs Patients receiving selegiline at the same time point after selegiline administration or MA use.

Statistical analysis was performed using Mann-Whitney test.

Table 17 Summary of the AM/MA ratio of patients and MA abusers at various times after selegiline administration and MA uses, respectivly

Time after exposure (hrs) / AM/MA ratio	2	4	6	8	20
Patients receiving selegiline	0.92* (0.10)	0.80* (0.08)	0.74* (0.07)	0.91* (0.10)	0.98* (0.14)
MA abusers	0.22* (0.04)	0.27* (0.06)	0.41* (0.05)	0.34* (0.06)	0.25* (0.04)

The data shown were mean (SEM).

*$P < 0.01$; MA abusers vs Patients receiving selegiline at the same time point after selegiline administration or MA use.

Statistical analysis was performed using Mann-Whitney test.

Assessment of the reliability of AM/MA cut-off value for differentiating patients receiving selegiline from MA abusers

Table 18.1 Determination the cut-off value at the ratio of AM/MA 0.40

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	72	23
Number of subjects predicted as abusers	3	74
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{72}{75}$ X 100 = 96%

Specificity of prediction (%) = $\frac{74}{97}$ X 100 = 76.28%

Accuracy of prediction (%) = $\frac{72 + 74}{75 + 97}$ X 100 = 84.88%

Sensitivity = probabiliity that the test says the patients receiving selegiline when in fact they did receive selegiline

Specificity = probabiliity that the test says the persons were abusers when in fact they were abusers

Accuracy/efficiency = efficiency of the test to give the true results of true positive value plus true negative values or true patients receiving selegiline and true abusers

Table 18.2 Determination the cut-off value at the ratio of AM/MA 0.45

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	70	21
Number of subjects predicted as abusers	5	76
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{70}{75}$ X 100 = 93.33%

Specificity of prediction (%) = $\frac{76}{97}$ X 100 = 78%

Accuracy of prediction (%) = $\frac{70 + 76}{75 + 97}$ X 100 = 84.88%

Table 18.3 Determination the cut-off value at the ratio of AM/MA 0.50

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	68	19
Number of subjects predicted as abusers	7	78
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{68}{75}$ X 100 = 90.66 %

Specificity of prediction (%) = $\frac{78}{97}$ X 100 = 80.41%

Accuracy of prediction (%) = $\frac{68 + 78}{75 + 97}$ X 100 = 84.88%

Table 18.4 Determination the cut-off value at the ratio of AM/MA 0.51

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	67	18
Number of subjects predicted as abusers	8	79
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{67}{75}$ X 100 = 89.33%

Specificity of prediction (%) = $\frac{79}{97}$ X 100 = 81.44%

Accuracy of prediction (%) = $\frac{67 + 79}{75 + 97}$ X 100 = 84.88%

Table 18.5 Determination the cut-off value at the ratio of AM/MA 0.52

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	65	17
Number of subjects predicted as abusers	10	80
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{65}{75}$ X 100 = 86.66%

Specificity of prediction (%) = $\frac{80}{97}$ X 100 = 82.47%

Accuracy of prediction (%) = $\frac{65 + 80}{75 + 97}$ X 100 = 84.30%

Table 18.6 Determination the cut-off value at the ratio of AM/MA 0.53

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	65	17
Number of subjects predicted as abusers	10	80
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{65}{75}$ X 100 = 86.66%

Specificity of prediction (%) = $\frac{80}{97}$ X 100 = 82.47%

Accuracy of prediction (%) = $\frac{65 + 80}{75 + 97}$ X 100 = 84.30%

Table 18.7 Determination the cut-off value at the ratio of AM/MA 0.54

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	64	17
Number of subjects predicted as abusers	11	80
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{64}{75}$ X 100 = 85.33%

Specificity of prediction (%) = $\frac{80}{97}$ X 100 = 82.45%

Accuracy of prediction (%) = $\frac{64 + 80}{75 + 97}$ X 100 = 83.72%

Table 18.8 Determination the cut-off value at the ratio of AM/MA 0.55

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	62	17
Number of subjects predicted as abusers	13	80
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{62}{75}$ X 100 = 82.66

Specificity of prediction (%) = $\frac{80}{97}$ X 100 = 82.47%

Accuracy of prediction (%) = $\frac{62 + 80}{75 + 97}$ X 100 = 82.55%

Table 18.9 Determination the cut-off value at the ratio of AM/MA 0.56

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	60	16
Number of subjects predicted as abusers	15	81
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{60}{75}$ X 100 = 80%

Specificity of prediction (%) = $\frac{81}{97}$ X 100 = 83.50

Accuracy of prediction (%) = $\frac{60 + 81}{75 + 97}$ X 100 = 81.97

Table 18.10 Determination the cut-off value at the ratio of AM/MA 0.57

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	59	15
Number of subjects predicted as abusers	16	82
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{59}{75}$ X 100 = 78.66%

Specificity of prediction (%) = $\frac{782}{97}$ X 100 = 84.53%

Accuracy of prediction (%) = $\frac{59 + 82}{75 + 97}$ X 100 = 84.53%

Table 18.11 Determination the cut-off value at the ratio of AM/MA 0.58

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	59	15
Number of subjects predicted as abusers	16	82
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{59}{75}$ X 100 = 78.66%

Specificity of prediction (%) = $\frac{82}{97}$ X 100 = 84.53%

Accuracy of prediction (%) = $\frac{59 + 82}{75 + 97}$ X 100 = 81.97%

Table 18.12 Determination the cut-off value at the ratio of AM/MA 0.59

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	59	15
Number of subjects predicted as abusers	16	82
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{59}{75}$ X 100 = 78.66%

Specificity of prediction (%) = $\frac{82}{97}$ X 100 = 84.53%

Accuracy of prediction (%) = $\frac{72 + 74}{75 + 97}$ X 100 = 81.97%

Table 18.13 Determination the cut-off value at the ratio of AM/MA 0.60

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	58	15
Number of subjects predicted as abusers	17	82
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{58}{75}$ X 100 = 77.33%

Specificity of prediction (%) = $\frac{82}{97}$ X 100 = 84.53%

Accuracy of prediction (%) = $\frac{58 + 82}{75 + 97}$ X 100 = 81.39%

Table 18.14 Determination the cut-off value at the ratio of AM/MA 0.65

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	53	12
Number of subjects predicted as abusers	22	85
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{53}{75}$ X 100 = 70.66%

Specificity of prediction (%) = $\frac{85}{97}$ X 100 = 87.62%

Accuracy of prediction (%) = $\frac{53 + 85}{75 + 97}$ X 100 = 87.62%

Table 18.15 Determination the cut-off value at the ratio of AM/MA 0.70

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	46	9
Number of subjects predicted as abusers	29	88
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{46}{75}$ X 100 = 61.33%

Specificity of prediction (%) = $\frac{88}{97}$ X 100 = 90.72%

Accuracy of prediction (%) = $\frac{46 + 88}{75 + 97}$ X 100 = 77.90%

Table 18.16 Determination the cut-off value at the ratio of AM/MA 0.75

Predicted status	Actual status	
	Patient	Abuse
Number of subjects predicted as patients	37	8
Number of subjects predicted as abusers	38	89
Total numbers of subjects	75	97

Sensitivity of prediction (%) = $\frac{37}{75}$ X 100 = 49.33%

Specificity of prediction (%) = $\frac{89}{97}$ X 100 = 91.75%

Accuracy of prediction (%) = $\frac{37 + 89}{75 + 97}$ X 100 = 73.25%

Table 19 Summary of assessment of the reliability of AM/MA cut-off value for differentiating patients receiving selegiline from MA abusers (data from Table 18.1-18.16)

AM/MA	Sensitivity (%)	Specificity (%)	Accuracy (%)
0.40	96.00	76.28	84.88
0.45	93.33	78.00	84.88
0.50	90.66	80.41	84.88
0.51	89.33	81.44	84.88
0.52	86.66	82.47	84.30
0.53	86.66	82.47	84.30
0.54	85.38	82.45	83.72
0.55	82.66	82.47	82.55
0.56	80.00	83.50	81.97
0.57	78.66	84.53	84.53
0.58	78.66	84.53	81.97
0.59	78.66	84.53	81.97
0.60	77.33	84.53	81.39
0.65	70.66	87.62	87.62
0.70	61.33	90.72	77.90
0.75	49.33	91.75	73.25

CHAPTER V

DISCUSSION AND CONCLUSION

The objective of this study was to compare the ratio of AM/MA concentrations in urines of Thai patients receiving selegiline therapy to the ratio of AM/MA concentrations in urines of Thai MA abusers. In addition, the possibility of using the ratio of AM/MA to preliminarily differentiate patients receiving selegiline therapy from MA abusers was also assessed. The study was conducted using the urine samples of patients at Prasat Neurological Institute, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand and the urine samples of MA abusers whose urines were sent to the Institute of Forensic Medicine, Police General Hospital. Bangkok, Thailand for forensic analysis.

Validation of the assay procedure for determining MA and AM concentrations in urines

In this study, MA and AM concentrations in urines were determined using SPME-GC-MS according to the method modified from Myung et al. (1998). This method is generally used in routine forensic toxicological analysis (Wang, 2005), which is not capable to differentiate the compounds with enantiomers such as *l*-MA and *l*-AM which are metabolites of selegiline from *d*-MA and *d*-AM which are excreted in urines of MA abusers. Before performing urinary MA and AM analysis, the assay procedure was validated according to the guidance suggested for analysis of compounds in biological sample (CDER and CVM, 2001). Linearity, precision of both within day and between day, and accuracy were tested. It was shown that urinary MA or AM concentrations were linearly correlated to the peak area ratio of MA or AM to internal standard. Within day and between day precision were shown by % CV of less than 15%. Accuracy as shown by % recovery was shown to be within 15%. These results were acceptable according to the recommendation that % CV determined at each concentration should not exceed 15% and the % recovery should be within 15% of the actual value (CDER and CVM, 2001).

MA and AM concentrations and the AM/MA concentration ratio in urine samples of patients receiving selegiline

Based on the narcotic regulation, a person with urine MA concentration of $\geq$1000 ng/ml is accused as illegal MA consumption. Thus, patients who receive selegiline either for therapeutic

purpose or overdosage, MA and AM which are metabolites of selegiline can be detected in their urines causing the false positive interpretation as MA users. Results from this study supported this particular concern. It was shown that high incidence (range 86.66-100%) of false positive interpretation of MA consumption was shown in patients receiving selegiline at therapeutic dosage regimens. Several previous studies demonstrated that the ratios AM/MA concentrations in urines of patients receiving selegiline are mostly higher than those of MA users suggesting that urinary AM/MA would be a helpful marker to distinguish selegiline patients and MA abuses (Meeker and Reynolds, 1990; Fugita et al., 1999; Hasegawa et al., 1999; Kupiec et al., 1999; Kim et al., 2000 and). Those studies were performed using urines of either deceased with selegiline overdose or healthy volunteers (Hasegawa et al., 1999), in Western countries and Japan. In this study, the subjects were Thai patients who were prescribed selegiline for therapeutic purposes. Most of the patients were old ages (63.53% ± 9.21 years) consistly to the pathological status of patients who were prescribed selegiline for Parkinson's disease. Their urine samples were collected at 5 time points after selegiline administration so as to assess the correlation between the AM/MA ratio and times after selegiline administration. No significant correlation was shown when selegiline was given twice daily while significant correlation was shown as selegiline was given once daily. This was consistent to the results reported by Hasegawa et al. (1999), that demonstrated the correlation between AM/MA and times after selegiline administration as once daily. The ratios of AM/MA gradually increased from 0.24 to 0.67 ($r = 0.857$) along with times (2-48 hours) after selegiline administration (Hasegawa et al., 1999). In contrast, when all the data of patients of all dosage regimens were assessed, correlation between AM/MA ratio and times after selegiline administration was not observed and the ratio seemed to be lower at 6 hours than at other times after selegiline ingestion. This could be due to effect of the second dose of selegiline that added more of the MA metabolite.

Concentrations of MA and AM in urines of MA abusers collected at various times after MA uses

Regarding the MA abusers included into this study, they were mostly male 91.75% and young age (28.46 ± 0.72 years). Due to the limitation of multiple urine collections in MA abusers after their lastest uses, the data of MA and AM concentrations as well as the corresponding AM/MA at each time point (2, 4, 6, 8 and 20 hours) after MA uses were not obtained from the

same persons as in the patients. MA concentrations in urine samples of most MA abusers were far higher than those of the patients and all were more than 1000 ng/ml. Thus, false negative interpretation of MA use were not shown in all MA abusers in this study. There was no correlation between AM/MA concentrations in urines of MA abusers and times after MA uses. Excretion ratios of MA were highest 3-6 after administration, Urinary MA Concentration peaked in the first 3 hours and remained increased through 12-24 hours while renal excretion of AM was saturable 12 hours after dosing (Oyler et al., 2002). Urinary AM/MA ratios of patients receiving selegiline were significantly higher than those of MA abusers at every corresponding time points after the compounds ingestion. This difference may be explained by the pharmacokinetic of the compounds. Selegiline is metabolized to AM via two pathways. One pathways yields AM while the other yields MA which is further metabolized to AM (Shin, 1997). Thus, higher AM/MA ratio is found in patients receiving selegiline. In contrast, in MA abusers, MA is excreted mainly unchanged in urine (30-50%) while less amount of AM (4-10%) is excreted in urine (Jirovsky et al., 1998; Moore, 1999). Thus, less AM/MA ratio is found in MA users.

An attempt to find the most reliable AM/MA cut-off value to differentiate patients receiving selegiline from MA abusers was performed with the values of between 0.40-0.75. This was because these values were between the lowest AM/MA of patients (0.74 ± 0.07) and the highest AM/MA of MA abusers (0.41 ± 0.05) found at 6 hours after selegiline administration and MA uses, respectively. The result showed that using the AM/MA ratio of 0.40 as the cut-off value resulted in the highest percentage of accuracy (84.88%). Also, using the cut-off value of 0.4 provided the highest probability that the patients receiving selegiline were predicted as patients and provided the lowest probability of patients to be predicted as abusers Thus, based on the results from this study, the AM/MA ratio of 0.40 is suggested to be preliminarily used as the cut-off value to differentiate selegiline users from MA abusers. However, this ratio is not an absolute marker for the conclusion. Further analysis is needed using the method which is enantioselective and sufficiently sensitive such as utilization of derivatizing reagent (Chang et al., 2001; Chiu et al., 2004; Tzing et al., 2006) or chiral column (Hasegawa et al., 1999; Kim et al., 2000) during the analysis by GC-MS (Frank et al., 1978; Konig and Benecke, 1981; Liu et al., 1982; Hasegawa, 1999; Chang et al., 2001; Chiu et al., 2004; Tzing et al., 2006) or LC-MS (Wainer and Doyle, 1983; Armstrong, 1987; Karnes and Sarkar, 1987; Pirkle et al., 1987).

In conclusion, results from this study showed that the ratio of AM/MA concentrations in urines of patients receiving selegiline therapy was significantly higher than the ratio of AM/MA concentrations in urines of MA abusers. The ratio of AM/MA could be preliminarily used to differentiate patients receiving selegiline from MA abusers with an accuracy of 84.88% when using the AM/MA ratio of 0.40 as the cut-off value.

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APPENDICES

APPENDIX A

Ethical Approval of Study Protocol

ที่ สธ ๐๙๑๐ (๑๒๕๐๐)/๒.๒๕๐

คณะกรรมการวิจัยสถาบันประสาทวิทยา
สถาบันประสาทวิทยา เลขที่ ๓๑๒ ถนนราชวิถี
แขวงทุ่งพญาไท เขตราชเทวี กรุงเทพฯ ๑๐๔๐๐

๒ มีนาคม ๒๕๕๔

เรื่อง อนุมัติให้ดำเนินการวิจัยได้

เรียน น.ส. นันทิกา แก้วปัญญา

ตามที่ท่านซึ่งเป็น หัวหน้าโครงการวิจัยตามรายละเอียดข้างท้าย ได้เสนอ โครงการวิจัยดังกล่าวต่อคณะกรรมการวิจัยสถาบันประสาทวิทยา

เลขที่โครงการ ๕๔๐๒๐๙
ชื่อโครงการ การเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจีลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย

ในการนี้ คณะกรรมการวิจัยสถาบันประสาทวิทยา ซึ่งเป็นคณะกรรมการวิจัยสถาบัน (Institutional Review Board : IRB) ที่มีการดำเนินงานตามแนวทางการวิจัยทางคลินิกที่ดี (ICH GCP) ได้พิจารณา และมีมติอนุมัติให้ดำเนินการโครงการวิจัยดังกล่าวในสถาบันประสาทวิทยาได้ โดยผู้วิจัยจะต้องมีหน้าที่และความรับผิดชอบภายหลังได้รับการอนุมัติ คือ ต้องปฏิบัติตามพระราชบัญญัติสุขภาพแห่งชาติ พ.ศ. ๒๕๕๐ มาตรา ๗ "ข้อมูลสุขภาพของบุคคล เป็นความลับส่วนบุคคล ผู้ใดจะนำไปเปิดเผยในประการที่น่าจะทำให้บุคคลนั้นเสียหายไม่ได้ เว้นแต่การเปิดเผยนั้นเป็นไปตามความประสงค์ของบุคคลนั้นโดยตรง" โดยเคร่งครัด และจะต้องรายงานความก้าวหน้าของโครงการวิจัยเมื่อมีการร้องขอและ/หรือเมื่อเกิดเหตุการณ์ต่อไปนี้ ทุกครั้ง ได้แก่

๑. เมื่อโครงการวิจัยยุติลง ซึ่งอาจจะเป็นการดำเนินการวิจัยเสร็จสิ้นสมบูรณ์ หรืออาจจะไม่สามารถดำเนินการวิจัยต่อไปได้ พร้อมทั้งแจ้งสาเหตุของการยุติโครงการวิจัยให้ทราบด้วย
๒. เมื่อมีการเปลี่ยนแปลงในโครงการวิจัยต้องระบุให้ชัดเจนว่า มีการเปลี่ยนแปลงอะไร อย่างไร พร้อมเหตุผลที่ต้องเปลี่ยนแปลง
๓. เมื่อมีการเปลี่ยนแปลงหัวหน้าโครงการวิจัยหรือเพิ่มเติมคณะผู้วิจัย ต้องส่งประวัติของคนที่เปลี่ยนแปลงพร้อมเหตุผลให้คณะกรรมการฯ ทราบด้วย
๔. เมื่อมีอาการไม่พึงประสงค์เกิดขึ้นในโครงการวิจัย ขอให้ผู้วิจัยวิเคราะห์สถานการณ์การเกิดอาการไม่พึงประสงค์ที่ relate, possible/likely, probably related, fatal กับโครงการวิจัยที่ท่านรับผิดชอบอย่างไร รวมทั้งขอทราบมาตรการในการดูแลป้องกันอาสาสมัครในประเทศไทยด้วย
๕. จัดส่งรายงานการศึกษาวิจัย จำนวน ๑ ชุด ให้แก่สำนักงานคณะกรรมการวิจัยสถาบันประสาทวิทยา เมื่อสิ้นสุดการดำเนินงาน

จึงเรียนมาเพื่อโปรดทราบ

ขอแสดงความนับถือ

(นายสุชาติ หาญไชยพิบูลย์กุล)
ประธานคณะกรรมการวิจัยสถาบันประสาทวิทยา

สำนักงานคณะกรรมการวิจัยสถาบันประสาทวิทยา
ศูนย์วิจัยสถาบันประสาทวิทยา
โทร. ๐๒-๓๕๘๙๐๗๖ ต่อ ๒๔๐๒ โทรสาร ๐๒-๓๕๘๙๓๙๙

APPENDIX B

Research subject information sheet &

Informed consent

เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย (Research Subject Information Sheet)

<u>เอกสารแนะนำสำหรับอาสาสมัคร</u>

1. โครงการวิจัย การเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย
2. ผู้วิจัย นางสาวนันทิกา แก้วปัญญา
   ตำแหน่ง นิสิตหลักสูตรเภสัชศาสตรมหาบัณฑิต สาขาวิชาเภสัชวิทยา คณะเภสัชศาสตร์ จุฬาลงกรณ์ มหาวิทยาลัย
3. สถานที่ปฏิบัติงาน ภาควิชาเภสัชวิทยาและสรีรวิทยา คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพฯ 10330
   หมายเลขโทรศัพท์ 0-2218-8325 โทรสาร 0-2218-8324
   โทรศัพท์ ที่บ้าน 0-2539-2474 (ติดต่อได้สะดวก 24 ชั่วโมง)
   เคลื่อนที่ 08-5812-0856 (ติดต่อได้สะดวก 24 ชั่วโมง)
4. เนื้อหาสาระของโครงการวิจัยและความเกี่ยวข้องกับอาสาสมัคร ได้แก่
   4.1 เหตุผลและความจำเป็นที่ต้องทำการศึกษาวิจัย

เมทแอมเฟตามีนเป็นยาเสพติดที่เป็นปัญหาสำคัญของประเทศไทยในปัจจุบัน การตรวจพิสูจน์ว่ามีการเสพยาเสพติดชนิดเมทแอมเฟตามีนหรือไม่ ทำได้โดยตรวจหาสารเมทแอมเฟตามีนและแอมเฟตามีนในปัสสาวะ การตรวจพบเมทแอมเฟตามีนและแอมเฟตามีนในปัสสาวะของบุคคลใดเป็นหลักฐานสำคัญที่แสดงว่าบุคคลนั้นได้รับหรือเสพสารเสพติดให้โทษประเภทที่หนึ่ง ซึ่งมีบทกำหนดโทษระบุไว้ในพระราชบัญญัติยาเสพติดให้โทษ พ.ศ. 2522

การตรวจด้วยวิธีการจำเพาะเพื่อหาสเตอริโอไอโซเมอร์ของเมทแอมเฟตามีนและแอมเฟตามีนในตัวอย่างปัสสาวะ เพื่อแยกผู้ต้องสงสัย/ผู้ต้องหาที่เสพเมทแอมเฟตามีนออกจากผู้ป่วยที่ได้รับยารักษาโรค เช่น ยาซีลีจิลีนที่มีเมแทบอไลท์เป็นลีโวเมทแอมเฟตามีน (*l*-methamphetamine) และ ลีโวแอมเฟตามีน (*l*-amphetamine) ที่ออกมาในปัสสาวะด้วยนั้น จะต้องใช้เครื่องมือที่ราคาแพง ขั้นตอนที่ยุ่งยาก ไม่เหมาะสมสำหรับงานวิเคราะห์ที่มีปริมาณมากมาย ในสภาวะของประเทศไทยขณะนี้ การหาค่าอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีน ในตัวอย่างปัสสาวะอาจนำมาใช้ในการคัดกรองผู้ต้องสงสัย/ผู้ต้องหาที่เสพเมทแอมเฟตามีนออกจากผู้ป่วยที่ได้รับยาซีลีจิลีน เพื่อพิจารณาตรวจยืนยันด้วยวิธีจำเพาะต่อไป

4.2 วัตถุประสงค์ของการศึกษาวิจัย

เปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีน ในปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีนเพื่อการรักษา กับอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในปัสสาวะของผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย

4.3 วิธีการศึกษาวิจัยโดยสังเขป

- ผู้วิจัยพบผู้ป่วยที่มาพบแพทย์ในคลินิกประสาทวิทยา สถาบันประสาทวิทยา คัดเลือกผู้ป่วยที่ได้รับการรักษาด้วยยาซีลีจิลีนมาแล้วเป็นระยะเวลาไม่ต่ำกว่า 7 วัน โดยประสานงานกับแพทย์เจ้าของไข้
- อธิบายรายละเอียดการวิจัยรวมทั้งสอบถามความยินยอมในการทำวิจัย
- ผู้ป่วยลงชื่อในหนังสือยินยอมร่วมการวิจัย
- เก็บตัวอย่างปัสสาวะในปริมาตร 30 มิลลิลิตร ที่เวลาประมาณ 2, 4, 6, 8 และ 20 ชั่วโมงหลังจากรับประทานยาซีลีจิลีน
- นำตัวอย่างปัสสาวะไปวิเคราะห์เพื่อหาอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในปัสสาวะของอาสมัครที่ได้รับยาซีลีจิลีนเพื่อการรักษา เปรียบเทียบกับอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในปัสสาวะของผู้ติดยาเสพติดชนิดเมทแอมเฟตามีน

4.4 ระยะเวลาที่อาสาสมัครต้องเกี่ยวข้องในการศึกษาวิจัย คือ

หลังจากที่ยินยอมเข้าร่วมการวิจัย ตลอดจนสิ้นสุดการวิจัย

4.5 ประโยชน์ที่คาดว่าจะเกิดขึ้นทั้งต่ออาสาสมัครและต่อต่อผู้อื่น

ได้ข้อมูลอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในปัสสาวะของผู้ป่วยชาวไทยที่ได้รับยาซีลีจิลีนเพื่อการรักษา และอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีน และเมทแอมเฟตามีนในปัสสาวะของผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย เพื่อใช้ในการคัดกรองผู้ป่วยที่ได้รับยาซีลีจิลีนเพื่อการรักษา ออกจากกลุ่มผู้ติดยาเสพติดชนิดเมทแอมเฟตามีน เพื่อพิจารณาตรวจยืนยันด้วยวิธีจำเพาะต่อไป

4.6 กรณีมีการรักษาหรือการตรวจวินิจฉัยตามมาตรฐาน หรือทางเลือกการตรวจวินิจฉัยอื่นๆ

ในกรณีของท่านจะเก็บตัวอย่างปัสสาวะของท่านในปริมาตร 30 มิลลิลิตร ที่เวลาต่างๆ เพื่อนำไปวิเคราะห์หาความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในปัสสาวะ

4.7 ขอบเขตการดูแลรักษาความลับของข้อมูลต่างๆของอาสาสมัคร

ผู้วิจัยจะเก็บข้อมูลเฉพาะเกี่ยวกับอาสาสมัครเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย หรือการเปิดเผยข้อมูลต่อผู้มีหน้าที่ที่เกี่ยวข้องกับการสนับสนุนและกำกับดูแลการวิจัย

4.8 กรณีเกิดอันตรายหรือผลไม่พึงประสงค์จากการศึกษาวิจัย

ในกรณีที่ท่านเกิดอาการไม่พึงประสงค์จากการใช้ยาซีลีจิลีน ท่านจะสามารถยุติการเป็นอาสาสมัครได้

4.9 การตอบแทนชดเชยแก่อาสาสมัคร

อาสาสมัครจะได้รับค่าตอบแทนท่านละ 250 บาท ต่อครั้ง

4.10 อาสาสมัครจะถอนตัวจากโครงการวิจัยได้ทุกเมื่อ โดยไม่กระทบต่อการดูแลรักษาที่พึงได้รับตามปกติ

4.11 กรณีมีเหตุจำเป็นหรือฉุกเฉิน อาสาสมัครสามารถติดต่อได้ทั้งในและนอกเวลาราชการได้ที่นางสาวนันทิกา แก้วปัญญา ที่อยู่ 338/101 หมู่บ้านกลางเมือง ถนนลาดพร้าว 80 เขตวังทองหลาง แขวงวังทองหลาง กทม. 10310 เบอร์โทรศัพท์ 08-5812-0856

ใบยินยอมด้วยความสมัครใจ

โครงการวิจัยเรื่อง การเปรียบเทียบอัตราส่วนระหว่างความเข้มข้นของแอมเฟตามีนและเมทแอมเฟตามีนในตัวอย่างปัสสาวะของผู้ป่วยที่ได้รับยาซีลีจิลีน และผู้ติดยาเสพติดชนิดเมทแอมเฟตามีนชาวไทย

วันให้คำยินยอม วันที่…….เดือน……..พ.ศ. ……

อาสาสมัครชื่อ.....

HN…….....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้น จนข้าพเจ้าพอใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้และเข้าร่วมโครงการวิจัยนี้โดยสมัครใจและการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะสรุปผลการวิจัย หรือการเปิดเผยข้อมูลต่อผู้มีหน้าที่ที่เกี่ยวข้องกับการสนับสนุนและกำกับดูแลการวิจัยเท่านั้น

บุคคลที่รับผิดชอบงานวิจัยนี้คือ นางสาวนันทิกา แก้วปัญญา ที่อยู่ 338/101 หมู่บ้านกลางเมือง ถนนลาดพร้าว 80 เขตวังทองหลาง แขวงวังทองหลาง กทม. 10310 หมายเลขโทรศัพท์ที่สามารถติดต่อได้ 24 ชั่วโมง 08-5812-0856

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม…….ผู้ยินยอม

( )

ลงนาม…….ผู้วิจัย

( )

ลงนาม…….พยาน

( )

ลงนาม……พยาน

( )

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจแล้ว และข้าพเจ้าจึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม……ผู้ยินยอม

( )

ลงนาม……ผู้วิจัย

( )

ลงนาม……พยาน

( )

ลงนาม……พยาน

( )

ในกรณีผู้ถูกทดลองยังไม่บรรลุนิติภาวะ จะต้องได้รับการยินยอมจากผู้ปกครองหรือผู้อุปการะโดยชอบด้วยกฎหมาย

ลงนาม……ผู้ปกครอง/ผู้อุปการะโดยชอบด้วยกฎหมาย

( )

ลงนาม……ผู้วิจัย

( )

ลงนาม……พยาน

( )

ลงนาม……พยาน

( )

ในกรณีที่ผู้ถูกทดลองไม่สามารถตัดสินใจเองได้ (โรคจิต-หมดสติ) ให้ผู้แทนโดยชอบด้วยกฎหมายหรือผู้ปกครอง หรือญาติที่ใกล้ชิดที่สุดเป็นผู้ลงนามยินยอม

ลงนาม……ผู้แทน/ผู้ปกครอง/ญาติ

( )

ลงนาม……ผู้วิจัย

( )

ลงนาม……พยาน

( )

ลงนาม……...พยาน

( )

BIOGRAPHY

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