Rasagiline Pharmacokinetics in CYP1A2 Variant Healthy Smokers and Non-smokers in Different Doses

Synopsis

PhD (Pharmacology)

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UNIVERSITYOF HEALTH SCIENCES, LAHORE
SYNOPSIS PROFORMA

Title of research project:

Rasagiline Pharmacokinetics in CYP1A2 Variant Healthy Smokers and Non-smokers in Different Doses

Synopsis submitted for: *PhD*	***Discipline:*** *Pharmacology*

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Qualifications:

M.Phil	2008	University of Health Sciences, Lahore
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Practical Experience:

- Currently employed as Associate Professor, Department of Pharmacology, CMH Lahore Medical & Dental College from 1st Jan. 2013 till date.
- As Assistant Professor of Pharmacology, Department of Pharmacology, CMH Lahore Medical & Dental College from 1stOct.2009 to 31st Dec 2012.
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Name of post-graduate institution: University of Health Sciences, Lahore.		
Name of Academic Supervisor Prof.Dr.Naseem Saud Ahmad	**Signature:**	**Date:**
Name of Head of Department Prof.Dr.Naseem Saud Ahmad	**Signature:**	**Date:**
Name of Principal/dean Maj.Gen.(R) Prof. Muhammad Aslam	**Signature:**	**Date:**
Convener, Ethical Review Committee **Approval letter attached** Prof.Dr.Muhammad Tahir	**Signature:**	**Date:**
Chairman (Advanced Studies & Research Board) Approved Not Approved **Vice Chancellor, UHS**	**Signature:**	**Date:**

ETHICAL DECLARATION

We undertake that,

We will abide by the declaration of World Medical Association (WMA) made at Helsinki (2008) regarding the ethical principles for medical research involving human subjects such as:

1. The health of the subjects will be prior consideration.
2. The procedures shall be explained to the subjects clearly and expressed consent shall be obtained.
3. All procedures shall be kept aseptic and painless.
4. The confidentiality of the information shall be assured and maintained.
5. Data shall be used for publication only.

The title of the research project shall be **"Rasagiline Pharmacokinetics in CYP1A2 Variant Healthy Smokers and Non-smokers in Different Doses".**

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ETHICAL REVIEW COMMITTEE

FOR MEDICAL AND BIOMEDICAL RESEARCH

University of Health Sciences Lahore, Pakistan

Dated:_____

The committee considers the ethical aspects of project titled "Rasagiline Pharmacokinetics in CYP1A2 Variant Healthy Smokers and Non-smokers in Different Doses" and undertaking by the investigator Dr.Rabiea Bilal supervised by Prof.Dr.Naseem Saud Ahmad and co-supervised by Prof.Dr.Sualeha Riffat and Dr.Shah Jahan, to observe the conditions, laid down in the declaration by World Medical Association at Helsinki (2008) regarding the ethical principles for medical research involving human subjects as:

1. The health of the subjects will be prior consideration.
2. The procedures shall be explained to the subjects clearly and expressed consent shall be obtained.
3. All procedures shall be kept aseptic and painless.
4. The confidentiality of the information shall be assured and maintained.
5. Data shall be used for publication only.

Further, the members pursued the project and were satisfied with the undertaking of the investigators.

Prof. Dr. Muhammad Tahir Chairman Ethical Review Committee University of Health Sciences Lahore	**Prof. A.H. Nagi** Member Ethical Review Committee University of Health Sciences Lahore	**Prof. Aslam Khan** Member Ethical Review Committee University of Health Sciences Lahore

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LIST OF ABBREVIATIONS

AE	Adverse effect
ANOVA	Analysis of variance
APO	Advanced Planner and Optimizer
AUC	Area under curve
BMI	Body mass index
CBC	Complete blood count
C_{max}	Maximum concentration in plasma
Cl_h	Hepatic clearance
Cl_r	Renal clearance
CYP1A2	Cytochrome P450 Type 1A2
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetraacetic acid
HPLC	High performance liquid chromatography
LFT	Liver function test
LID	Levodopa induced dyskinesia
MAO-B	Monoamine oxidase –Type B
MAO-B I	Monoamine oxidase Type B inhibitor
PCR	Polymerase chain reaction
PD	Parkinson's Disease
PDA	Photo diode array
RFLP	Restriction fragment length polymorphism
RFT	Renal function test
RM	Rasagiline mesylate
RPM	Revolution per minute
SNpc	Substantia Nigra pars compacta
SPSS	Statistical package of social sciences
$t_{1/2}$	Plasma half life
T_{max}	Time to reach maximum concentration
UV	Ultra violet
Vd	Volume of distribution

LIST OF APPENDICES

Project Summary:

Rasagiline a propargylamine, is a second generation inhibitor of enzyme monoamine oxidase-B (MAO-B) involved in endogenous breakdown of dopamine. The drug leads to increased levels of dopamine and is prescribed as monotherapy or in combination with levodopa for management of Parkinson's disease. Rasagiline is metabolized by cytochrome P450 type 1A2 (CYP1A2) enzyme encoded by CYP1A2 gene. This gene shows considerable interindividual variability for pharmacokinetics of its substrates. Genetic, dietry and environmental factors like smoking regulates the activity of CYP1A2.

In previous studies, it has been reported that for the drugs metabolized through this enzyme, the genotype variant A/A shows low plasma concentration as compared to A/C & C/C genotype. Tobacco smoking acts as an inducer of CYP1A2 enzyme. Increase in dose of the drug may be required to achieve the desired response of the drug in fast metabolizer variant (A/A) of this gene. This can prevent labeling these individuals resistant to this drug. Many clinical studies have used pharmacokinetic variables as the outcome measure for clinical response. Identification of these factors may be helpful in choosing an appropriate dosage of this drug improving efficacy & response in polymorphic and smoker patients of Parkinson's disease in our community.

In our study we will be comparing the pharmacokinetics of rasagiline among the three variants A/A,A/C & C/C of CYP1A2 drug metabolizing enzyme in smokers & non-smokers in three different doses. The study will be divided into two phases.

In first phase we will genotype the healthy volunteers to identify the three variants of CYP1A2 (A/A, A/C and C/C). Subjects (n=108) will be sub-grouped into smokers and non-smokers. Pharmacokinetic studies will be performed by giving them a single oral dose of rasagiline 1mg, 2mg & 5mg after an overnight fast of 10hrs. Serial blood sampling will be done over next 12hrs at intervals of 0, 0.25, 0.30, 1,2,4,6, 8, 10 & 14 & 18hrs. Blood will be centrifuged and plasma will be separated for HPLC analysis of pharmacokinetic parameters.The pharmaco-kinetic variables of rasagiline in all the groups will be compared using analysis of variance (ANOVA) and for intergroup comparisons with the Post hoc Tukey test. Data will be analysed using SPSS version 22.0 and statistical significance will be considered at $p<0.05$.

The study will be distinctive as to our knowledge, no such comparison of pharmacokinetic variables has been reported earlier to show the influence of genetic and smoking status on rasagiline in different doses. This work will endorse the need of dose adjustment under these conditions.

INTRODUCTION/LITERATURE REVIEW:

Parkinson's Disease (PD) is a neurodegenerative disorder affecting about 1% of the total population over the age of 60 and 4% over the age of 80years (Ahmed et al., 2014). The most common features of PD are resting tremor, rigidity, bradykinesia and postural instability due to degeneration of the dopaminergic neurons in nigrostriatal pathway originating from the substantia nigra pars compacta (SNpc). Non motor symptoms like decline in cognition, sleep disturbances, anxiety & depression appear progressively and require timely effective treatment (Aminoff, 2015). Drugs therapy includes levodopa, monoamine oxidase B inhibitors (MAO-B I), catechol-o-methyl-transferase inhibitors, dopamine agonists and anticholinergic drugs (Connolly and Lange, 2014).

Levodopa is still the drug of choice for managing PD symptoms, however there is reported incidence of 9-80% of levodopa-induced dyskinesias (LID),"ON-Off " effect, and other motor complications (Thanvi and Robinson, 2007). There is 50% higher incidence of LID with early age onset (40-49 years) of PD (Sossi et al. 2006).

Rasagiline, a propargylamine is a second generation MAO-B I. It is used as monotherapy or in combination with levodopa. In treatment of PD, the drug has shown promising results in improving daily motor activities and maintaining effect in longer run (Pagonnaparraqa and Rodriqu-Oroz, 2013). Literature reveals that Rasagiline shows improvement in total unified Parkinson's disease rating scale (UPDRS) for more than 30% as compared to placebo in patients of Parkinson's disease (Stern et al., 2004).

Rasagiline shows dose dependent pharmacokinetics. Thébault et al.(2004) has reported that the drug is safe and tolerable in doses of 1-20mg and there is proportional increase in C_{max} (maximum concentration in plasma) and AUC (area under curve) with increase in dose of drug. Bioavailability of rasagiline is 35% and it shows linear absorption in doses 1-10mg (Lecht et al., 2007). Pharmacokinetics of rasagiline 1mg in PD patients has shown C_{max} 8.5ng/ml, T_{max} 0.5-0.7hrs, Vd 182-243L, $t_{1/2}$ 1.34 hrs and mean oral clearance 94.3 L/hr (Chen and Anh-Vuong, 2006).

Table 1: Pharmacokinetics of rasagiline in healthy volunteers

Parameter	1mg	2mg	5mg	Reference
C_{max}	^2.5ng/ml #4.52ng/ml	^4.9ng/ml *9.5ng/ml	*26.4ng/ml	#Konda et al (2012) ^Lecht et al (2007) *Thébault et al (2004)
T_{max}	^0.5-1hrs #0.41	^0.5-1hr *0.36hrs	*0.51hrs	
AUC_{0-24} $AUC_{0-infinity}$	#4.02ng.hr/ml	*5.8ng.hr/ml	*18.6ng.hr/ml	
$t_{1/2}$	^1.5-3.5hrs #1.07	^1.5-3.5hrs		
Vd	^87-243L	^87-243L		
Cl_h	^94.3L/h			
Cl_r		*1.11L/hr	*0.35L/hr	

Where C_{max} =maximum observed drug concentration, T_{max} =time to reach maximum drug concentration, AUC_{0-24}=area under plasma concentration time curve during 24hrs,$AUC_{0-infinity}$= drug area under the concentration time-curves from time zero to infinity, $t_{1/2}$ =mean half life, Vd=volume of distribution, Cl_h= volume of blood perfusing the liver that is cleared of the drug per unit of time, $Cl_{r=}$ volume of plasma from which drug is completely removed by the kidney per unit of time.

Rasagiline is metabolized by hepatic CYP1A2 enzyme into 1-R-Aminoindan followed by its excretion through kidneys (Agúndez et al., 2013). The drug metabolizing enzyme CYP1A2 shows polymorphism (Womack et al., 2012)

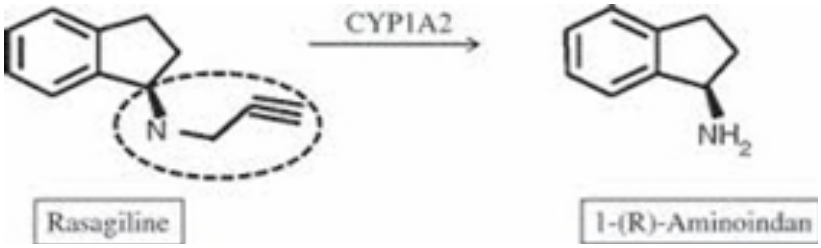

Fig.1 Biotransformation of rasagiline (Chen and Anh-Vuong, 2006).

The impaired hepatic status, genetic variations and co-administration of drugs with rasagiline may change the AUC and C_{max} of drugs. Rasagiline co-administered with ciprofloxacin caused 83% increase in AUC which required 50% dose reduction (Lecht et al., 2007; Chen & Anh-Vuong, 2006).

Genetic variability in drug metabolizing enzymes is considered as one of the most important determinant of variability in drug response (Yasuda et al., 2008). Clinical studies have used pharmacokinetic variables as their outcome measure (Li-wan-Po et al., 2009).

CYP1A2 gene is coded by a 7.8-kb gene, consisting of seven exons and six introns, mapped to chromosome 15. So far, 36 allelic variants have been identified (Faber et al., 2005; Rasmussen et al., 2002). A considerable interindividual variability, because of both genetic mutations and environmental factors has been observed in drugs metabolized by CYP1A2. It is highly inducible both in terms of enzyme activity and its genetic expression (Dobrinas, et al. 2013).

Smoking is a potent CYP1A2 enzyme inducer resulting in significantly lower plasma concentrations of the drugs (Van der Weide et al., 2003). The identification of genetic and environmental factors regulating the expression and activity of CYP1A2 may be helpful to rationalize the dose of drug. CYP1A2 -163C>A (rs762551) SNP is called

CYP1A2*1F and it characterizes the variant A/A (Sachse et al., 2001). There is C to A transition in intron 1 of the CYP1A2 gene at position 734 downstream of the first transcribed nucleotide. According to the human cytochrome P450 (CYP) Allele Nomenclature Committee, allele A is highly inducible. (Laika et al., 2010). CYP1A2*1F allele is quite common in the general population. The frequency of this variant is 0.68 in Caucasians & Egyptian and 0.61 in Japanese population (Hamdy et al., 2003). The mutation 163C>A appears to be associated with an increase in enzyme activity (Djordjevic, 2010). The frequency of rs762551:C > A varies widely with populations: the C allele frequencies range from 0.3 to 0.39 in Asians, from 0.4 to 0.51 in Blacks or African Americans, and from 0.29 to 0.33 in Whites (Thorn et al., 2012). According to Ensemble genome web browser, in Punjabi population of Lahore (PJL), the prevalence of allele A is 54% and C is 45% for rs762551 with prevalence of A/A 29%, A/C 50% & C/C 21% (Population genetics, 2015). CYP1A2*1F has been reported to show higher enzyme inducing activity in smokers (Pilgrim et al., 2012; Sachse et al., 2001).

In Swedish smokers rs762551 (A/A) was found to be associated with increased metabolism of caffeine, the probe drug for this enzyme. Smokers carrying homozygous C-allele have 40% lower CYP1A2 activity as compared to A/A variants (Thorn et al., 2012).

Several clinical studies have been conducted to examine the impact of CYP1A2 polymorphisms on the clearance of drugs and on the clinical response related to them. Resistance to clozapine associated with low plasma levels of the drug has been reported in smoker schizophrenic patients carrying the CYP1A2*1F (Olsson et al., 2015). In a study population taking olanzapine treatment, CYP1A2*1F with A/A variant resulted in a 22% reduced serum concentrations of the drug (Laika et al.,

2010). In another study it was reported that the dose of clozapine or olanzapine should be reduced to half in non-smokers than smokers due to higher inducibility of CYP1A2 enzyme for which both the drugs are substrate (Olsson et al., 2015).

Rasagiline has shown dose dependent effects on pharmacokinetics in a study conducted on healthy volunteers in single oral dose of 1mg, 2mg, 5mg & 10mg (Kim and Borton, 2010). There is no difference in tolerability of drug in younger and older patients (Perz-Lloret and Rascol, 2011). This drug is well tolerated with a few reported side effects of sleep disturbances, confusion, dry mouth, nausea, dizziness, anxiety & orthostatic hypotension (Robottom, 2011).

Our study is designed to observe the pharmacokinetics of rasagiline in healthy CYP1A2 variants (A/A,A/C and C/C). Smoking, a common CYP1A2 enzyme inducer is also under study in our program.

HYPOTHESIS

There is difference in mean pharmacokinetics of rasagiline in A/A, A/C & C/C variants of CYP1A2 somkers & non smokers.

Aims & Objectives:

1) Rasagiline drug extraction & HPLC method validation in human plasma.
2) Genotyping of CYP1A2 variants A/A, A/C & C/C for pharmacokinetic study.
3) Comparison of pharmacokinetics of rasagiline among CYP1A2 variants A/A, A/C & C/C in different doses.
4) Comparison of pharmacokinetics of rasagiline between smokers & non-smokers in different doses.

Rationale:

The rationale of the study is to identify the genetic and smoking factors which may effect the pharmacokinetics of rasagiline.

SUBJECTS & METHOD

Study design

Comparative, Interventional, single oral dose, pharmacokinetic study

Setting

The study will be carried out in Department of Pharmacology University of Health Sciences Lahore (UHS) and University of Veterinary & Animal Sciences, Lahore (UVAS).

Duration of study

The study will be carried out in about one and a half years' time after approval of synopsis.

Sample size

Given that the goal of this project is to study the pharmacokinetics of rasagiline in CYP1A2 variants, a target sample size of 108 healthy volunteers for pharmacokinetic study is selected. (Stern et al., 2004; Thébault et al., 2004).

Sampling technique

It is purposive sampling.

Sample selection

Healthy volunteers will be enrolled from different universities of Lahore. They will be reasonably compensated for participation in study. The study protocol will be confirmed by the ethical committee of UHS & UVAS in compliance with Good Clinical Practice and the Declaration of Helsinki.

Inclusion criteria

Healthy volunteers of both sexes with age between 18 to 30 years and Body Mass Index <30 (BMI=weight/height^2) will be included in the study.

Smoking will be defined as present if a participant reports to be smoking at the time of survey either daily or occasionally. Non-smoker is a person who does not smoke at all or at the time of survey (W.H.O,1998). Cotinine urine dip test kit will be used to differentiate between smokers & non-smokers (Paek et al., 2009).

Exclusion Criteria

Volunteers with unstable medical condition or deranged CBC, LFT & RFT

Volunteers with history of drug allergies

Volunteers who have received any medication which is substrate for CYP1A2
Volunteers who have donated blood within 2 months

Pregnant women

Data Collection

Subjects meeting the inclusion & exclusion criteria will be provided with the forms for informed consent (Appendix I).

Socio-demographis information, personal & family medical history will be taken (Appendix II).

Clinical examination and laboratory investigations will be done (Appendix III).

Safety assessment and blood sampling will be carried out according to the schedule in Appendix IV.

Tables showing pharmacokinetic variables are attached in Appendix V.

METHODOLOGY

Genotyping

For the determination of genotype method used by Sachse et al. (2001) will be taken as reference. 5 ml of whole blood will be collected in EDTA tubes by venipuncture. The whole blood will be stored at 4^0C until use for DNA extraction.

DNA Extraction

DNA will be extracted using a DNA kit.

DNA Amplification by PCR

Genotyping of CYP1A2*1F alleles C-163A will be done by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique. For detection of CYP1A2 variants the region flanking each variant will be amplified in PCR using a specific set of primers. Then a suitable genotyping technique Taqman assay will be employed to ascertain the genotypes of each variant. Standard protocol will be adopted for amplification of corresponding DNA fragments by PCR and relevant genotyping technique.

Grouping of subjects

Smokers and non-smokers CYP1A2 variants will be randomly grouped by lottery method for dosing of rasagiline. The enrolled healthy volunteers (n=108) will be grouped as follows:

Table 2: Grouping of CYP1A2 variant healthy smokers & non-smokers

	A/A		**A/C**		**C/C**	
Dose	Smokers	Non-smokers	Smokers	Non-smokers	Smokers	Non-smokers
1mg	6	6	6	6	6	6
2mg	6	6	6	6	6	6
5mg	6	6	6	6	6	6

Treatment protocol

Participants will be asked to avoid smoking, coffee, tea, carbonated beverages, grape fruit juice, chocolate or any caffeine- containing drinks or medicine night before and during the pharmacokinetic study. Female volunteers will be asked to abstain from hormonal contraceptives for a minimum of 3 weeks prior to study.

Drug intervention

After an overnight fast of 10 hrs tab.rasagiline 1mg, 2mg & 5mg will be given with 240 ml of water as designed in table 2.

Blood Sampling

All the participants will be housed in a big hall with comfortable environment. Standardized meals will be served to the subjects during the study period (Appendix

IV). Aseptic techniques will be used to collect blood samples which will be collected by performing venipuncture and putting indwelling cannula for 12 hrs. Five milliliter of whole blood will be collected in EDTA tubes using at 0 hr before medication and then at 0.25, 0.5, 1, 2, 4, 6, 8, 10,14 & 18hrs after medication (Appendix IV). The blood samples will be centrifuged at 4000 rpm for 30 min. Plasma will be separated from the blood samples and preserved at -80^0 C until analyzed.

Safety Assessments: The safety assessments include adverse effects of rasagiline, vital signs and clinical examinations. Monitoring of AEs will be conducted throughout the study and observations will be recorded at the times specified in the Schedule of Events (Appendix IV).

Experimental

Method developed by Ravi et al. (2012) using high performance liquid chromatography (HPLC) with ultra violet (UV) detector will be followed.

Chemicals and reagents

Rasagilines (Searle Pharmaceutical Karachi, Pakistan) will be procured from local pharmacy store. Ammonium acetate, glacial acetic acid, sodium citrate, acetonitrile and methanol of HPLC-grade will be procured from the manufacturers. A water purification system will be used to obtain high quality water. Ultrafiltration will be carried out using centrifugal filters.

Instruments

A liquid chromatographic system with auto injector and UV detector (Agilent 1260 LC, USA) will be used. C18 column, vortex mixer , refrigerated centrifuge and deep freezer will be required for preparation and processing of samples in method development and validation. Filteration of aqeous phase will be carried out using

membrane filters.

Method development

In the process of HPLC method development for rasagiline, mobile phase composition and flow rate will be optimized by experimenting with different aqueous phase and non-aqueous phase combinations at different flow rates. Buffers with different strengths and in varying compositions with acetonitrile and/or methanol will be investigated.The mobile phase composition and flow rate will be finally selected based on the criteria of peak properties (Ravi et al., 2012).

Method validation

The developed method will be validated according to standard guidelines. Limit of detection & quantification, Linearity, Precision & Accuracy, Recovery and Stability will be evaluated.

Pharmacokinetic Parameters:

Following pharmacokinetic parameters will be calculated by entering plasma concentration-time data in available software.

C_{max}

T_{max}

AUC

$t_{1/2}$

Vd

Cl

STATISTICAL ANALYSIS:

Data will be entered and analyzed using latest version of Statistical Packages for Social Sciences (SPSS) for windows. Mean ± S.D. (Standard Deviation) will be calculated for quantitative variables of pharmacokinetics and 2-way ANOVA (Analysis of Variance) will be applied for intergroup comparisons.

For all statistical tests, p-value equal to or less than 0.05 will be considered statistically significant.

OUTCOME & UTILIZATION:

We will be revalidating HPLC-UV method for measuring concentration of rasagiline in human plasma in our setup for further pharmacokinetic studies related to drug interactions and polymorphism of different enzymes and transporters to which the drug is a substrate.

Similar pharmacokinetic pattern of rasagiline with different doses will augment the involvement of our proposed variables.

This work will endorse the need of identification of smoking and genetic screening of CYP1A2 variants, A/A, A/C & A/C for drug dose optimization leading to proper management of Parkinson's patients.

PLAN OF WORK

2016-2017

	Apr	Jun	Aug	Oct	Dec	Feb	Apr	Jun	Aug	Oct
Lit. Survey										
Genotyping										
PK sampling										
Analysis of data										
Thesis writing										

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Appendix 1

CONSENT FORM

University of Health Sciences Lahore, Pakistan

Rasagiline pharmacokinetics in CYPIA2 variants healthy smokers and non smokers in different doses

I _____,CNIC no._____ hereby, fully agree to contribute in the above mentioned study. I understand that the study is designed to add to the knowledge of Medical Science. I have been informed about the nature of the procedure and the possible risks/discomfort involved. I was given the opportunity to ask any query about the study and I agree to give blood sample to DR.RABIEA BILAL, the researcher as requested. I have no objection in case the data obtained from this investigation is published in research publication, maintaining confidentiality.

میں _____ شناختی کارڈ نمبر_____ مکمّل طور پر مندرجہ
بالا تحقیق میں ڈاکٹر ربیعہ بلال کے ساتھ تعاون کا اقرار کرتی ہوں۔ مجھے معلوم ہے کہ یہ تحقیق طبی علم میں اضافے کے لئے کی جارہی ہے۔ مجھے اس کے بنیادی طریقہ کار سے آگاہ کر دیا گیا ہے۔ اس تحقیق کے متعلق سوال جواب کرنے کا موقع دیا گیا ہے اور اس سے متعلق شکوک و شبہات دور کر دیے گئے ہیں۔ میں اس تحقیق کے لئے خون کا نمونہ دینے کے لئے تیار ہوں۔ اور مجھے اس چیز پر اعتراض نہیں ہے کہ میرے متعلق یہ تحقیق کسی رسالے میں بغیر ذاتیات کھولے شائع ہوگی۔

Signature:_____ **Date: _____**

Name:_____

CNIC No: _____

Contact No: _____

Appendix II

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF HEALTH SCIENCES LAHORE
Address: Khayaban-e-Jamia Punjab, Lahore 54000 Tel. :(042) 111 333 366
In collaboration with
BIOEQUIVALENCE STUDY (BeSt) CENTRE
UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES, LAHORE
Tel. 3 042-9211374, 042-9211449-50 (Ext. 287) Fax No. 042-9211461

Source Document 1: <u>Biodata and Medical History</u>

Clinical Investigator: _____

Subject number/enrolment code: _____

Screening Doctor:_____

Date: _____

Please complete these forms to the best of your ability and in black pen.
The quality of the study depends upon the quality of your answer.

Sur Name		First Name(s)	
Home address			
Contact No.			
email address			
Alternative contact methods			
Emergency contact detail			
		Phone no:	
Doctor or GP		Phone no:	

1. **Personal Details**

1.1 Date of birth: _____

1.2 Age: _____

1.3 Sex: **Male**_____ **Female**_____

1.4 Smoking: I am a smoker since_____

Daily smoker _____

Occasional smoker _____

Ex-smoker _____

Ex-Occasional _____

Never smoked _____

1.5 Drug use:

Do you use recreational drugs (e.g Ectasy, cocaine) or have you used drugs for recreational purpose in the past? Yes_____, No_____

1.6 Alcohol consumption: Yes_____, No_____

1.7 Blood donation:

Have you donated blood within the last 2 months? Yes____, No_____

Date of most recent donation _____

1.8 Drug Trials

Have you ever participated in a clinical trial involving

Investigational medicines before? Yes_____, No_____

Date of most recent participation _____

2. Medical Details

2.1 Have you ever suffered from asthma? Yes_____, No_____

2.2 Have you ever suffered from eczema? Yes_____, No_____

2.3 Have you ever suffered from Hay fever? Yes_____, No_____

2.4 Have you ever been diagnosed with tuberculosis?Yes_____, No_____

2.5 Have you ever experienced an allergic reaction?Yes_____, No_____

2.6 Are you allergic to any drug or medication? Yes_____, No_____

2.7 Have you ever had anything wrong with your kidneys? Yes_____, No_____

2.8 Have you ever had anything wrong with your liver? Yes_____, No_____

2.9 Have you ever had hepatitis or jaundice? Yes_____, No_____

2.10 Have you ever suffered from heartburn? Yes_____, No_____

2.11 Have you ever had anything wrong with your stomach or intestine?
Yes_____, No_____

2.12 If you have answered yes to any of the above, please supply the relevant detail.

_____

_____

2.13 If you suffer from any other condition, please supply details.

_____

_____

WOMEN ONLY:

2.14 Are you married? Yes_____, No_____

2.15 Are you currently menstruating? Yes_____, No_____

2.16 When was your last menstrual period? Date:_____

2.17 Are you pregnant? Yes_____, No_____

2.18 Are you currently breastfeeding a baby? Yes_____, No_____

2.19 If you are using a form of contraception, please specify below:

_____

_____

_____

3. Medical and Surgical Details

3.1 Have you undergone any surgery during the last 12 month?

Yes_____, No_____

If yes, please give the details below: Date:_____

Reason:

_____

_____

3.2 Have you fallen ill during the last 12 month?

Yes_____, No_____

If yes, then please give the details below:

Onset: _____End: _____

Details of illness:

_____

_____

4. Declaration

I, _____, NIC no._____hereby declare that all the information provided above is correct.

Signed:_____ Date: _____

Name: _____Place: _____

Supervising Clinician:

Signed:_____ Date: _____

Appendix III

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF HEALTH SCIENCES LAHORE
Address: Khayaban-e-Jamia Punjab, Lahore 54000 Tel. :(042) 111 333 366
In collaboration with
BIOEQUIVALENCE STUDY (BeSt) CENTRE
UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES, LAHORE
Tel. Off. 92-42-9210256 Ext, 287 Fax # 042-9213303

Deputy Director's Office

Source Document 2: **Clinical Examination & Lab Investigation**

Clinical Investigator: _____

Subject number/enrolment code: _____

Date: _____

Please complete these forms to the best of your ability and in black pen.

Laboratory investigations

CBC, Creatinine, Urea, AST, ALT

B-HCG (Women)

Urinary Ph, Protein, glucose, RBC

1. Subject Details

Weight _____
Height _____
Age _____

2. Systemic Enquiry

Recent weight loss_____
Recent weight gain_____
Malaise_____
Night sweats _____
Fever_____
Wheeze_____
Chest pain _____
Palpitations _____
Dyspnoea _____
Headaches _____
Oedema_____
Gastrointestinal problem_____
Urinary symptoms_____

3. Vital Signs

Blood pressure Systolic_____
Diastolic_____

Temperature _____^{o}C

Pulse rate _____

Respiratory rate _____

4. Examination

a. General

Jaundice Yes_____, No_____
Pallor Yes_____, No_____
Cyanosis Yes_____, No_____
Oedema Yes_____, No_____
Lymphadenopathy Yes_____, No_____

b. Systemic:

Cardiovascular_____

Chest and lungs _____

Abdomen _____

Nervous system_____

Musculoskeletal_____

Skin and mucosae_____

Eyes and ears_____

Others_____

Venous access Poor_____ Adequate_____

Appendix IV

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF HEALTH SCIENCES LAHORE
Address: Khayaban-e-Jamia Punjab, Lahore 54000 Tel. :(042) 111 333 366
In collaboration with
BIOEQUIVALENCE STUDY (BeSt) CENTRE
UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES, LAHORE
Tel. Off. 92-42-9210256 Ext, 287 Fax # 042-9213303

Volunteer ID:_____
Protocol No: _____
Protocol Title: RASAGILINE PHARMACOKINETICS IN CYP1A2 VARIANT HEALTHY SMOKERS & NON-SMOKERS IN DIFFERENT DOSES

Safety Assessment:

VITAL SINGS

Date	**RR**			**Temperature**			**Pulse Rate**			**Blood Pressure**		
	Time	Breaths/min	Sign	Time	^{o}F	Sign	Time	Beats/min	Sign	Time	mmHg	Sign
	08:30											
	09:30											
	11:00											
	1:00											
	15:00											
	19:00											
	23:00											
	03:00											

Clinical examination for side effects of rasagilne:

All the volunteers will be monitored for the following side effects that may occur with rasagiline:
Dizziness, drowsiness, joint pain, heartburn, nausea, fever, muscle pain, dry mouth, and stomach/abdominal pain.

Stopping rules:
Volunteer will be withdrawn from the study and managed promptly if any of the following serious side effects appear:

Dangerously high blood pressure (severe headache, blurred vision, buzzing in ears, anxiety, confusion, chest pain, shortness of breath, uneven heartbeats, seizure)
Sudden numbness or weakness (especially on one side of the body), problems with speech or balance
Unusual thoughts or behavior, agitation, hallucinations, fever, fast heart rate,

overactive reflexes,vomiting, diarrhea, loss of coordination, fainting, tremor, muscle twitching or stiffness, feeling like he might pass out.

BLOOD SAMPLING FORM

Date DD MM YY Day 1	**Sample ID**	**Activity**	**Sample Collection Time**			**Signature Sampler**	**Signature Supervisor**
			Time (hrs)	**Proposed**	**Actual**		
	$1BA_o$	Sampling	-0.5	07:30			
	Dosing (with water-250 ml)		00.00	08:00			
	$1BA_1$	Sampling	0.25	08:15			
	$1BA_2$	Sampling	0.50	08:30			
	$1BA_3$	Sampling	01.00	09:00			
	$1BA_4$	Sampling **Breakfast**	02.00	10:00 10:15			
	$1BA_5$	Sampling	04.00	12:00			
	$1BA_6$	Sampling **Lunch**	06.00	14:00 14:30			
	$1BA_7$	Sampling	08.00	16:00			
	$1BA_8$	Sampling	10.00	18:00			
	IBA_9	Sampling	14.00	22:00			
		Dinner	14.30	22:30			
	IBA_{10}		18.00	02:00			

Name of sampler:_____ Signature:_____

Name of supervisor:_____

Appendix V

TABLES:

Mean Pharmacokinetics of Rasagiline at 1mg, 2mg & 5mg in ***smokers*** in CYP1A2 variants:

	A/A			A/C			C/C		
	1mg	2mg	5mg	1mg	2mg	5mg	1mg	2mg	5mg
C_{max}									
T_{max}									
AUC									
$T_{1/2}$									
Vd									
CL									

Pharmacokinetics of Rasagiline at 1mg, 2mg & 5mg in ***non-smokers*** in CYP1A2 variants:

	A/A			A/C			C/C		
	1mg	2mg	5mg	1mg	2mg	5mg	1mg	2mg	5mg
C_{max}									
T_{max}									
AUC									
$T_{1/2}$									
Vd									

CL									