DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HALOPERIDOL AND REDUCED HALOPERIDOL IN PLASMA: APPLICATION IN PHARMACOKINETIC STUDY

by

THEEPA ASUALINGAM

Thesis submitted in fulfillment of the requirement for the Degree of

Master of Science

April 2007

To my beloved Sidha Yogi Siva Sangkara baba, parents, husband, son, parents-in-law and sisters.

ACKNOWLEDGEMENTS

First and foremost, I wish to express my utmost gratitude and heartfelt appreciation to my dedicated principal supervisor, Professor Sharif Mahsufi Mansor for his guidance, invaluable advice and encouragement throughout my graduate program. I am most grateful for all the scientific knowledge I have gained from him and all the fruitful discussions we have had. I would like to thank my co-supervisor, Dr. Surash Ramanathan for always being so enthusiastic about the projects I have been involved in and for his endless support throughout my studies.

I am also taking this opportunity in thanking Professor Yuen Kah Hay for providing his support and lab facility and Dr. Ng Bee Hong for her expert technical assistance during animal study. Thanks also to the Janssen foundation for providing reduced haloperidol. I am grateful to the Institute of Graduate Studies, USM for providing the financial assistance through the Graduate Assistance Scheme during my candidature.

I would specially like to thank Mr. A.K.M. Mahbubuzzaman for being with me throughout and sharing my joys and disappointments. Not forgetting also my dear friends Kanabathy, Shubatra Pillay, Lai Choon Shen, G. Venkatesh, Chitra and Venessa Daniel. I would also like to acknowledge my gratitude to all the staff and lab assistants of CDR, especially to Mr R. Arunachalam and Mr. M.Asokan.

My deepest gratitude goes to my parents for their continuous encouragement, support and interest throughout my studies. Very special thanks to my husband, Mr. N. Ragu for his patience, constant encouragement, unconditional love as well as financial and moral support during my research.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATION	Full name
ACE	Animal Ethics Committee
ACN	Acetonitrile
AGP	Alpha-1-acid glycoprotein
AHFS	American hospital formulary services
A_s	Asymmetry factor
AUC_{0-12}	Area under the curve from time zero to 12 h.
$AUC_{0-\infty}$	Area under the curve from time zero to infinity.
AUFS	Absorption units full scale
BP	British Pharmacopoeia
C18	Column having octadecyl chain of C atom
C8	Column having octadecyl chain of C atom
CL	Clearance
C_{max}	Peak plasma concentration
CV	Coefficient of Variation
CYP	Cytochrome P_{450}
CYP2D6	Cytochrome P_{450} subfamily 2D6
CYP3A4	Cytochrome P_{450} subfamily 3A4
D_2	Dopamine subtype receptor 2
et al.	Co-workers
FDA	Food and Drug Administration
F-T	Freeze-thaw
g	Gram
h	Hour
HP	Haloperidol

HPLC	High Performance Liquid Chromatography
HPLC-EC	High Performance Liquid Chromatography coupled with electrochemical detector
HPLC-UV	High Performance Liquid Chromatography coupled with ultraviolet detector
ICH	International Conference on Harmonization
IS	Internal standard
k’	Capacity factor
K^+	Potassium ion
KH_2PO_4	Potassium dihydrogen phosphate
l	Liter
LLOQ	Lower limit of quantification
LLE	Liquid-liquid extraction
LOD	Limit of detection
M	Molar
MeOH	Methanol
ml	Milliliter
mm	millimeter
mM	millimolar
MRT	Mean residence time
N	Normality
n	Number of replicate
Na^+	Natrium ion
NaOH	Sodium hydroxide
ng	Nanogram
nm	nanometer
ODS	Octadecylsilane
pH	negative logarithm of H^+ concentration
pKa	Ionisation constant

PYR	Pyrimethamine
r	Correlation coefficient
RH	Reduced haloperidol
RP	Reverse phase
RP-HPLC	Reversed-phase high performance liquid chromatography
rpm	revolution per minute
R_s	Resolution
SD	Standard deviation
Sec	Second
S.E.M.	Standard error of the mean
SPE	Solid phase extraction
$t_{1/2}$	Elimination half-life
T_{max}	Time to reach peak plasma conentration
USP	United State Pharmacopoeia
UV	Ultraviolet
v/v	Volume by volume
v/w	Weight by volume
Vd	Volume of distribution
%	Percent
$\pm$	Plus/minus
α_1	Adenergic receptor subtype 1
μl	Microliter
μm	Micrometer
<	Less than
>	Greater than

PERKEMBANGAN DAN PENGESAHAN KAEDAH KCPT BAGI PENENTUAN HALOPERIDOL DAN HALOPERIDOL TERTURUN SECARA SERENTAK DI DALAM PLASMA : APLIKASI DALAM KAJIAN FARMAKOKINETIK

Abstrak

Haloperidol merupakan suatu drug antipsikotik yang tipikal dan secara kimianya daripada kumpulan butirofenon. Suatu kaedah kromatografi cecair berprestasi tinggi yang peka dan selektif dengan pengesan ultra-lembayung telah diperkembangkan bagi penentuan haloperidol dan haloperidol terturun secara serentak di dalam plasma. Drug dikesan pada 230 nm. Pemisahan kromatografi dilakukan dengan menggunakan turus KCPT Inertsil C8-3 (150 x 4.6 mm, 5μm). Fasa bergerak yang digunakan terdiri daripada 50 mM larutan penimbal fosfat (pH 5.0) – metanol (51:49, v/v) dengan kadar aliran fasa bergerak 1.0 ml/min. Pengekstrakan cecair-cecair yang mudah telah dihasilkan dan pirimetamina digunakan sebagai piawai dalaman. Purata peratus pengembalian bagi haloperidol, haloperidol terturun dan pirimetamina adalah 82.4, 82.1 dan 82.0% masing-masing. Kaedah ini menunjukkan selektiviti yang baik di mana tidak terdapat gangguan daripada puncak-puncak drug antipsikotik yang lazim digunakan. Keluk kalibrasi adalah linear bagi julat kepekatan 1-60 ng/ml dengan pekali korelasi (r) > 0.999. Peratus pekali variasi bagi kepersisian kaedah dalam sehari dan hari ke hari adalah kurang daripada 5%. Had pengesanan dan had kuantifikasi bawah adalah 0.5 ng/ml dan 1.0 ng/ml masing-masing bagi haloperidol dan haloperidol terturun.

Stok piawai yang dilarutkan dalam metanol didapati stabil selama tiga bulan pada suhu -20°C. Manakala sampel-sampel plasma didapati stabil selama 6

bulan pada suhu-20°C and -85°C Kaedah ini telah diaplikasi dengan jayanya dalam kajian farmakokinetik haloperidol di dalam tikus di mana haloperidol diberikan secara dos oral (2.5 mg/kg). Nilai purata $t_{1/2}$, C_{max}, t_{max}, $AUC_{(0-\infty)}$, CL dan Vd adalah 6.2 ± 2.6 h, 24.9 ± 7.2 ng/ml, 2.0 ± 1.9 j, 214.1 ± 76.0 ng.j/ml, 213.0 ± 61.9 ml/min/kg dan 110.3 ± 46.3 l/kg masing-masing. Kaedah analisis bagi haloperidol dan haloperidol terturun yang telah dihasilkan didapati sesuai bagi kajian farmakokinetik.

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HALOPERIDOL AND REDUCED HALOPERIDOL IN PLASMA: APPLICATION IN PHARMACOKINETIC STUDY

Abstract

Haloperidol is a typical antipsychotic drug that chemically belongs to butyrophenone group. A sensitive and selective reversed-phase HPLC method with ultraviolet detection was developed for the simultaneous determination of haloperidol and reduced haloperidol in plasma. Drugs were detected at 230 nm. The chromatographic separation was performed on an Inertsil C8-3 (150 x 4.6mm, 5 μm) HPLC column. Mobile phase composed of 50 mM phosphate buffer pH 5.0 and methanol (51:49, v/v) and eluted at 1.0 ml/min. A simple liquid-liquid extraction was carried-out using pyrimethamine as an internal standard. The mean extraction recovery for haloperidol, reduced haloperidol and pyrimethamine were 82.4, 82.1 and 82.0% respectively. The method showed good selectivity with respect to commonly administered psychotropic drugs. Calibration curve was linear over the concentration range 1 - 60 ng/ml with the correlation coefficient (r) > 0.999. The within-day and day-to -day assay precision was less than 5%. Limit of detection and lower limit of quantification were 0.5 ng/ml and 1 ng/ml respectively for haloperidol and reduced haloperidol.

Stock standard of haloperidol and reduced haloperidol in methanol were stable up to three months at -20°C. Plasma samples spiked with haloperidol and reduced haloperidol was stable up to 6 months at -20°C and -85°C. The method was successfully applied to pharmacokinetic study of haloperidol in rats following oral dose of haloperidol (2.5 mg/kg). The mean $t_{1/2}$, C_{max}, t_{max}, $AUC_{(0-\infty)}$, CL and Vd were 6.2 ± 2.6

h, 24.9 ± 7.2 ng/ml, 2.0 ± 1.9 h, 214.1 ± 76.0 ng.h/ml, 213.0 ± 61.9 ml/min/kg and 110.3 ± 46.3 l/kg respectively. The analytical method for haloperidol and reduced haloperidol assay is found to be suitable for pharmacokinetic studies.

CHAPTER 1
INTRODUCTION

1.1 PSYCHOSIS

Psychosis is a mental disorder which involves striking disturbances of thought, perception, affect, and behaviour (Pantelis *et al*., 2003). The expression of psychotic symptoms varies over time and across patients, however the cumulative effects of the illness are always severe and usually long lasting (Sadock & Sadock, 2000). There are various types of psychotic disorders such as anxiety, bipolar disorder (manic disorder), depression and schizophrenia (Grilly, 1994). These disorder a briefly discussed in the following sections.

1.1.1 ANXIETY

Anxiety is a normal response to psychological stress induced by either physical or perceived threat. However, a malfunctioning anxiety response could lead to anxiety disorders. Anxiety disorders are further divided into five types; such as panic disorder, social phobia, obsessive compulsive disorder, generalised anxiety disorder and posttraumatic stress disorder (Leveleki *et al*., 2006; Ballenger, 1999). Patients suffering from anxiety disorder are usually treated either by medication or psychotherapy (Ballenger, 1999).

1.1.2 MANIA OR BIPOLAR DISORDER

Mania or bipolar disorder is a brain disorder that causes unusual shifts in a person's mood, energy and ability to function. During a manic episode, the mood disturbances are severe enough to cause significant impairment in occupational functioning or in otherwise normal social activities (Schapiro, 2005). Bipolar disorder is further classified into two groups namely Bipolar I and Bipolar II. Bipolar I is characterised by one or more manic or mixed episodes. Bipolar II is characterised by

recurrent episodes of major depression and hypomania. Bipolar disorder affects approximately three to five percent of the world's population, and affects both sexes equally in all age group (Shastry, 2005).

1.1.3 DEPRESSION

Depression is defined as a feeling of intense sadness. Depressed individuals tend to be obsessed with personal failings, are apathetic and socially withdrawn (Malatynska & Knapp, 2005). Depression disorder can be transmitted in the form of negative emotions from living or non-living beings to susceptible host. Therefore, depression is also known as communicable disorder (Kalra, 2004). Symptoms of depression disorder can last periods of time, sometimes several to years (Gard, 2001).

1.1.4 SCHIZOPHRENIA

Schizophrenia is a complex cognitive disorder comprising of a variety of alterations in attention, working memory, language, response monitoring and inhibition (Freedman, 2003). Schizophrenia is treated as a debilitating disorder of the central nervous system. Its symptoms are divided into two classes namely positive symptoms and negative symptoms. Positive symptoms include hallucinations, delusions, conceptual disorganization, where negative symptoms include social withdrawal, blunted affect, and poverty of speech (Sawa & Snyder, 2002; Donaldson *et al*., 1983). This disorder reduces the ability of the individual to interact with the social. Schizophrenia affects about 1% of the world's population (Buchanan & Carpenter, 2000). All these types of mental disorders mentioned above are treated with antipsychotic drug that will be described in the following section.

1.2 ANTIPSYCHOTIC DRUG

The term antipsychotic is applied to a group of drugs used to treat psychosis. These classes of drugs were originally called 'neuroleptics' (from the Latin root which mean to grasp the neuron) (Nicholas, 2004). Antipsychotic drugs are generally divided in two types; that is typical antipsychotic drug and atypical antipsychotic drug.

1.2.1 TYPICAL ANTIPSYCHOTIC DRUG

Typical antipsychotics are also known as major tranquilizers because of their tranquilising and sedating effects when taken in large doses. Typical antipsychotic drugs are effective primarily against positive symptoms of schizophrenia. Such drugs include haloperidol (HP), chlorpromazine, perphenazine, thioridazine and trifluoperazine are examples of typical antipsychotic drug (Fig. 1.1). Chlorpromazine was the first typical antipsychotic drug used in 1952 to treat schizophrenic patients (Edliner *et al*., 2005). Antipsychotic properties of typical antipsychotics are achieved through the antagonistic effect on dopamine receptors.

The typical antipsychotic drugs used to treat schizophrenia are highly effective. In particular, HP, the most widely used typical antipsychotic is very efficient in treating the positive symptoms of schizophrenia. The most predominant among these symptoms are dystonia, parkinsonian-like syndrome, and tardive dyskinesia (Andreassen *et al*., 1996).

1.2.2 ATYPICAL ANTIPSYCHOTIC DRUG

Atypical antipsychotic drugs are used to treat schizophrenic patients and results less extrapyramidal side effects. Compared to the older ‘typical’ antipsychotic, the atypical antipsychotic drugs are equally effective against the positive symptoms and the negative symptoms of schizophrenia. Atypical antipsychotic drugs

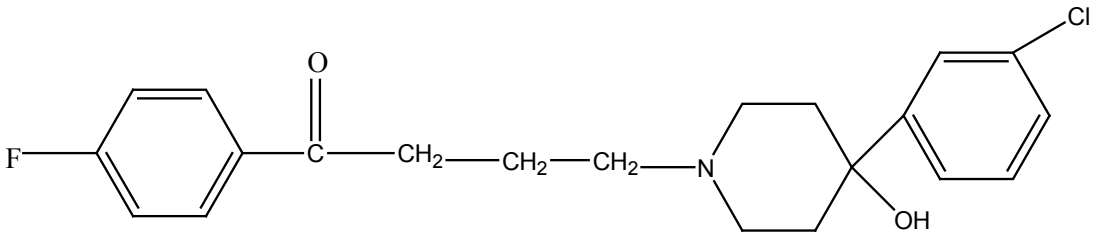
block both dopamine and serotonin (5-hyroxytryptamine) receptors. These combined effects on both dopamine and serotonin (5-hyroxytryptamine) receptors explain the lower extrapyramidal side effects of atypical antipsychotic drugs. Examples of atypical antipsychotic drugs are ariprazole, risperidone, clozapine, olanzapine, quetiapine and ziprasidone (Fig. 1.2).

1.3 HALOPERIDOL

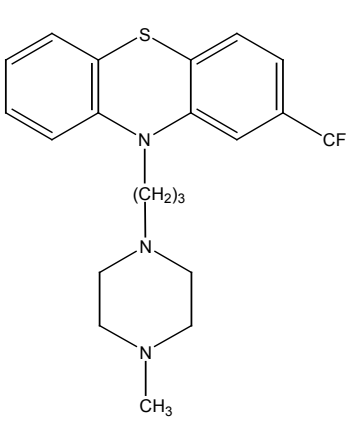
HP is {4-[4-(p-chlorophenyl)-4-hydroxypiperidino] 4'-flurobutyrophenone} a typical butyrophenone antipsychotic drug. HP is a tertiary amine that occurs as a white or almost white powder, is practically insoluble in water, and is slightly soluble in alcohol, methanol (MeOH) and methylene chloride. The melting point of HP is 150 to 153°C. The empirical formula for HP is $C_{21}H_{23}ClFNO_3$ (B P, 2003). The drug has a pKa value of 8.3 (AHFS, 2000).

HP is effective in the treatment of many psychotic disorders such as hyperactivity, agitation and mania (Robert & Allain, 2001). HP effectively treats positive symptoms of schizophrenia (Glick *et al*., 2001) while ineffective against negative symptoms of schizophrenia. HP is also used in the treatment of neurological disorders such as Gilles de la Tourette syndrome, Huntington's chorea and acute/chronic brain syndrome (Silver *et al*., 2001; Barr *et al*., 1988; Maltbie & Cavenar, 1977). Long-term use of HP can result in side effects resembling Parkinson's disease and tardive dyskinesia, an irreversible motor disorder (Fuxe *et al*., 1989).

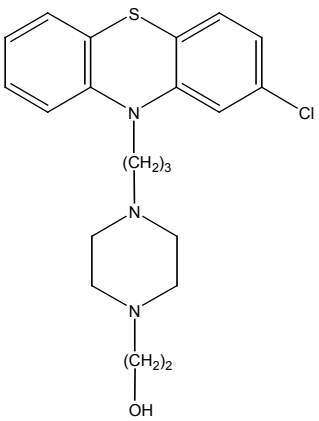
Nearly 40 years after its discovery, HP is still one of the most popular drug used for the management of various classes of psychosis. The pharmacology of HP has been extensively reported (Janssen, 1967). Different assay methods have been described in the literature, which will be discussed in the following section.



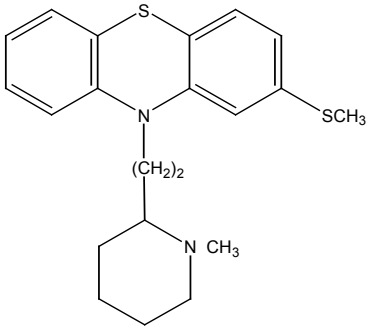
a. Haloperidol



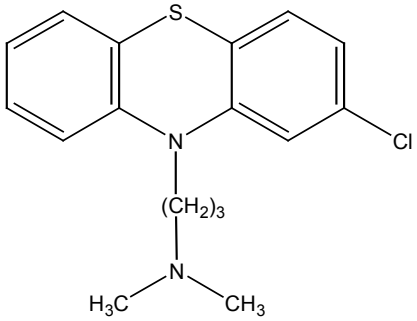
b. Trifluoperazine



c. Perphenazine

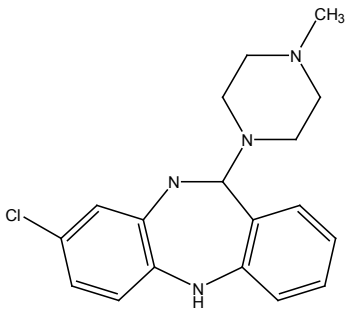


d. Thioridazine

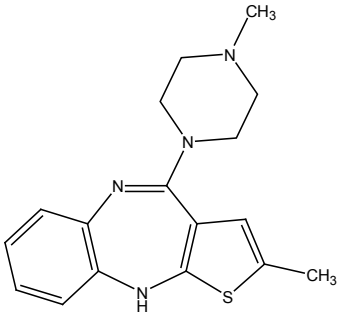


e. Chlorpromazine

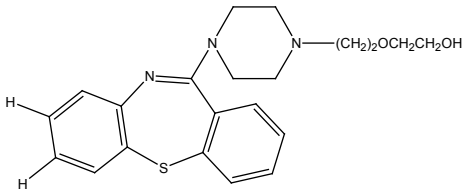
Figure 1.1 Structural formula of typical antipsychotic drugs.



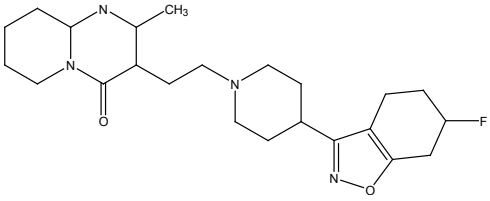
a. Clozapine



b. Olanzapine



c. Quetiapine



d. Risperidone

Figure 1.2 Structural formula of atypical antipsychotic drugs.

1.3.1 ASSAY OF HP AND RH IN BIOLOGICAL FLUIDS

Several analytical methods have been reported in the literature for the determination of either HP or RH or both drugs in biological fluids. All these methods involve various types of detection techniques (Table 1.1). Selection of a detection technique depends on the types of analytes, and the sensitivity required. For routine analysis, the most commonly used detection techniques are ultraviolet (UV) and electrochemical (EC) methods.

A number of techniques are available in the literature for the determination of HP in plasma. Some of these include gas chromatography (Bianchetti & Morselli, 1978), radioimmunoassay (Clark *et al*., 1977) and UV methods (Kogan *et al*., 1983; Miyazaki & Arita, 1981). Pharmacological studies showed that reduced haloperidol (RH), metabolite of HP is pharmacologically active. Therefore, a routine method for determination of plasma level should include both HP and RH. Simultaneous determinations of HP and RH in human plasma by HPLC system coupled with UV (Miller & Devane, 1986; Cahard *et al*., 1990; Park *et al*., 1991) or EC (Aravagiri *et al*., 1994; Korpi *et al*., 1983; Eddington & Young, 1988) method have been reported.

Although HPLC with EC detection is highly sensitive, it involves the use of high voltage that can be problematic in monitoring HP plasma levels (Parkinson, 1985). Furthermore, increased interference from co-administered drugs is observed in assay (Pan *et al*., 1998). Several other methods reported in the literature include gas chromatography with nitrogen-phosphorus or electron-capture detector (Bianchetti & Morselli, 1978; Franklin, 1980; Forsman *et al*., 1974) and radioimmunoassay (Clark *et al*., 1977; Browning *et al*, 1985), are relatively time consuming and not a common routine method in many therapeutic drug monitoring laboratories. The

radioimmunoassay method is very cumbersome and lack specificity and sensitivity and with it, RH is determined by an indirect method.

Recently, liquid chromatography in combination with mass spectrometric detection (Hempenius *et al*., 1999; Hoja *et al*., 1997) has been reported for the analysis of HP. Sophisticated detector such as mass spectrometry coupled with liquid chromatography or gas chromatography is admittedly highly sensitive. However, it is very expensive and requires great expertise in order to operate. With such considerations in mind, a method based on UV detection is still the most practical for routine applications and therefore is the method of choice in this thesis.

For analysis of drugs and its metabolites in plasma, sample preparation is very important. HP and RH were extracted from biological fluids either by liquid-liquid extraction (LLE) or solid phase extraction (SPE) (Table 1.2). However, LLE is the most popular and convenient method for routine analysis of HP and RH determinations (Korpi *et al*., 1983; Miller & Devane, 1986; Hariharan *et al*., 1989; Park *et al*., 1991). Only a few groups used the SPE method (Hempenius *et al*., 1999; Hoffman & Edkinds, 1994; Cahard *et al*., 1990). LLE extraction involves single, double or multiple step of extraction. Extraction efficiency partly depends on the types of extraction solvents, sample preparation pH, back extraction solvents and physiochemical properties. Different types of extraction solvents are used to extract drug from plasma. In most case mixtures of organic non-polar and polar solvents are employed. For HP and RH determinations commonly used non-polar solvents are pentane, hexane and heptane and polar solvents are isoamyl alcohol and isopropanol (Table 1.2). However, from the reported recovery data presented in Table 1.2 it is difficult to conclude which combination of extraction solvents were the best, since extraction performance depend on several factors.

To keep drugs in a neutral state, basifying agents are generally added to plasma. Sodium hydroxide (Hariharan *et al*., 1989; Miller & Devane, 1986; Jatlow *et al*., 1982) and sodium carbonate (Aravagiri *et al*., 1994; Midha *et al*., 1988) at different concentrations were used as basifying agents for HP determination in plasma.

Different types of solvents are used to back extract the drug from organic phase into aqueous phase. For HP and RH determination hydrochloric acid (Midha *et al*., 1988; Miller & Devane, 1986; Hoja *et al*., 1997), perchloric acid (Hariharan *et al.,* 1989) and sulfuric acid (McBurney & George, 1984; Park *et al*., 1991) were used as back extraction solvents. However, organic acid (perchloric acid and acetic acid) are the preferred back extraction solvent as compared to inorganic acid (hydrochloric acid and sulphuric acid). A much cleaner blank chromatogram was obtained using perchloric acid rather than hydrochloric acid or sulphuric acid (Hariharan *et al*., 1989).

1.3.2 PHARMACOLOGY OF HP

A pharmacological action of drug is dependent upon its chemical structure, any change in chemical structure may change the drug action. The exact drug action also depends on its binding affinity to the receptor types. Drugs combine with these receptors to produce their pharmacological effect (Gard, 2001).

Pharmacological activity of HP is related to its affinity for the dopamine receptors. (Beuger *et al.,* 1996; Creese *et al.,* 1976; Seeman & Lee, 1975; Seeman *et al*., 1974). Dopamine receptors are classified into five subtypes such as D_1, D_2, D_3, D_4 and D_5 (Dearry *et al*., 1990; Grandy *et al*., 1991; Monsma *et al*., 1990; Sokoloff *et al*., 1990; Sunahara *et al*., 1991; Vantol *et al*., 1991). These receptors are further divided into two subfamilies such as the D_1-like receptors (D_1 and D_5) and the D_2-like receptors (D_2, D_3 and D_4)(Tarazi, 2001). The binding of HP to the postsynaptic D_2-like receptors

is believed to mediate the therapeutic effects (Creese *et al*., 1976; Seeman & Lee, 1975; Seeman *et al*., 1974). In addition to the specific functions of several subtypes, dopamine receptors also have distinct functions based on their location at the neuron, i.e. pre-synaptically or post-synaptically (Nagy *et al*., 1978). Post-synaptic dopamine receptors are necessary for signal initiations or transmission, whereas pre-synaptic dopamine receptors, located on the cell bodies and axon terminals, function as autoreceptors through which the release of dopamine can be regulated.

HP, which is a dopamine antagonist, mimics the dopamine in binding to the dopamine receptor. There appears to be a very narrow range between the effective therapeutic dose for the management of acute psychotic disorder and that, which causes extrapyramidal side effects (AHFS, 2000). Therefore, the determination of antipsychotic drugs plasma concentration in psychiatric patient is important, especially in case of the HP, due to large variability between individuals. When HP is metabolised in the liver it produces the metabolite RH. This reduced metabolite, RH is then oxidised back to HP. Therefore, the influence of HP and RH on clinical responses requires the monitoring of plasma levels for better patient management during HP therapy.

Table 1.1 Summary of analytical method for HP determination in biological matrix.

References	Detector	Internal standard	Column	Mobile phase	Extraction method	LOQ/ LOD	Recovery (%)
Miyazaki & Arita. (1981)	HPLC-UV 250 nm	Diphenylamine	C18 Nucleosil 250 x 4 mm, i.d. 5μm	Methanol-0.2 M ammonium acetate (63:37, v/v)	LLE	5 ng/ml (LOD)	HP :102%
Jatlow *et al*. (1982)	HPLC-UV 195 nm	Desipramine	C18 Bio-sil ODS 250 mm i.d. 10 μm	Acetonitrile-Potassium dihydrogen phosphate pH3.8-4 (40:60, v/v)	LLE	1 ng/ml (LOD)	HP :78%
Kogan *et al*. (1983)	HPLC-UV 254 nm	Chlorohaloperidol	C18 Absorbosphere 100 x 4.6 mm, i.d. 3 μm	Acetonitrile-0.4 ml concentrated sulfuric acid, 1.1 g tetramethyl ammonium chloride, 0.5 ml triethylamine per liter (45:55, v/v)	LLE	2 ng/ml (LOD)	HP :80%
Korpi *et al*. (1983)	HPLC-EC	Chlorohaloperidol	μBondapak CN 300 x 3.9 mm, i.d. 10 μm	Acetonitrile-potassium phosphate buffer pH 6.8 (45:55, v/v)	LLE	0.5 ng/ml (LOD)	HP:92% RH:93%
Dhar & Kutt. (1984)	HPLC-UV 254 nm	Chloro-substitute analog	C8 bonded RP 250 x 4.6 mm, i.d. 5 μm	Acetonitrile-methanol-16.5mm disodium hidrogen phosphate pH5 (45:25:30, v/v/v)	LLE	1 μg/l (LOD)	HP:89%
Miller & Devane. (1986)	HPLC-UV 196 nm	Chlorohaloperidol	C18 250 x 4.6 mm, i.d. 10 μm	Acetonitrile-100 mm monobasic potassium phosphate pH 3.8-4 (55:45, v/v) flow rate : 2.0 ml/min	LLE	2 ng/ml (LOD)	HP:86% RH:92%

Table 1.1 continued

References	Detector	Internal standard	Column	Mobile phase	Extraction method	LOQ/ LOD	Recovery (%)
Eddington & Young. (1988)	HPLC-EC	Chlorohaloperidol	Nitrile bonded LC-PCN 150 x 4.6 mm, i.d. 5 μm	Acetonitrile-1-propanol-10 mm dipotassium hidrogen phosphate pH 6.8 (30 :20 :50, v/v/v) flow rate : 1.1ml/min	SPE	20 pg (LOD)	HP :93% RH :90%
Hariharan *et al*. (1989)	HPLC-coulometri	Bromoperidol	C8 RP (DB) 250 x 4.6 mm, i.d. 5 μm	Acetonitrile-methanol-50 mM KH_2PO_4 pH 7 (20:40:40, v/v/v)	LLE	50 ng/l (LOD)	HP :83% RH :76%
Cahard *et al*. (1990)	HPLC-UV 220 nm	Chlorohaloperidol	C18 μBondapak 300 x 3.9 mm, i.d. 10 μm	Acetonitrile-water-0.025 M potassium dihydrogenphosphate (45:5:50, v/v/v) flow rate 0.8 ml/min	SPE	1 ng/ml (LOQ)	
Wilhelm & Kemper. (1990)	Photo-diode	Clonazepam	Silica 100 RP-18 120 x 4 mm, i.d. 5 μm	Gradient : ethanol-acetonitrile-0.05 M phosphate buffer pH2.9 (22:8:70, v/v/v)	SPE	450 pg/ml (LOQ)	HP :101%
Park *et al*. (1991)	HPLC-UV 214 nm	Bromoperidol	C18- Nova Pak 150 x 2.9 mm, i.d. 4 μm	Acetonitrile-methanol-0.5 M potassium dihydrogen phosphate (31:11:58, v/v/v/) Flow rate : 0.6 ml/min	LLE	0.5 ng/ml (LOD)	HP :95% RH :73%
Aravagiri *et al*. (1994)	HPLC-EC	Chlorohaloperidol	Ultrasphere Cyano 250 x 4.6 mm, i.d. 5 μm	Acetonitrile-methanol-0.04M ammonium acetate pH 6.8 (86:6:8, v/v/v)	LLE	0.1 ng/ml (LOQ)	HP :68% RH:86%

Table 1.1 continued

References	Detector	Internal standard	Column	Mobile phase	Extraction method	LOQ/ LOD	Recovery (%)
Hoffman & Edkins. (1994)	HPLC-coulometri	Chlorohaloperidol	Microsorb – 100 x 4.6 mm, i.d. 3 μm	Methanol-50mM sodium phosphate pH 6.5 (50:50, v/v) Flow rate : 0.5ml/min	SPE	0.5 ng/ml (LOD)	HP :80%
Hoja *et al*. (!997)	LC-EC-MS	Chlorohaloperidol	C18 Nucleosil 150 x 1 mm, i.d. 5 μm	Acetonitrile-0.04M ammonium formate (45:55, v/v)	LLE	0.075 ng/ml (LOD)	HP :58% RH:70%
Pan *et al*. (1998)	HPLC-UV 220 nm HPLC-EC	Chlorohaloperidol	C18 Symmetry 150 x 3.9 mm, i.d. 5 Altima CN 250 x 4.6 mm, i.d. 5 μm	Acetonitrile-0.02M sodium dihydrogen phosphate pH 3.8 (29:71, v/v) Acetonitrile-Methanol-0.04M sodium dihydrogen phosphate pH 6.8 (35:20:45, v/v/v)l	LLE	2 ng/ml (LOQ) 0.5 ng/ml (LOQ)	HP :99-104% HP :83-121%
Hempenius *et al*. (1999)	LC-MS	Haloperidol D4	C18 Symmetry 100 x 4.6 mm, i.d. 3.5 μm	Methanol-0.2% formic acid (50:50, v/v)	SPE	0.1 (LLQ)	
Titier *et al*. (2003)	HPLC-UV 240 nm	Methylrisperidone	C8 Symmetry i.d. 250 x 4.6 mm,	Gradient : Acetonitrile-50mM NaH_2PO_4	LLE	5 ng/ml (LOQ)	HP:69%

Abbreviation: HPLC-UV = High performance liquid chromatography-Ultraviolet detector; HPLC-EC= High performance liquid chromatography-electrochemical detector; LC-EC-MS= liquid chromatographic-electrospray mass spectrometric; LC-MS= liquid chromatographic mass spectrometric; i.d.= internal diamension; mm=milimeter; μm=mikrometer; nm=nanometer; LLE = liquid-liquid extraction; SPE= solid phase extraction; LLOQ=lower limit of quantification, LOQ=limit of quantification; LOD=limit of detection; HP=haloperidol; RH=reduced haloperidol.

Table 1.2 Summary of extraction method for HP determination in biological matrix.

References	Extraction solvent	Basifying agent	Back extraction solvent	Reconstitute solvent
Titier *et al*. (2003)	Hexane : isoamylalcohol (99:1, v/v) (7 ml)	Sodium hydroxide (2 N, 100 µl))	Hydrochloric acid(0.05 M, 200 µl)	Direct injection
Pan *et al*. (1998)	Hexane : isoamylalcohol (100:2, v/v) (5 ml)	Sodium hydroxide (2 N, 200µl)	Hydrochloric acid (0.1 N, 150 µl)	Mobile phase
Hoja *et al*. (1997)	Hexane : isoamylalcohol (99:1, v/v) (8 ml)	Sodium hydroxide (2M, 500µl)	Hydrochloric acid (0.2 M, 1ml)	Mobile phase (50 µl)
Aravagiri *et al*. (1994)	Pentane:methylchloride (90:10, v/v) (7 ml)	Saturated sodium carbonate (0.5 ml)		Acetonitrile (150µl)
Park *et al*. (1991)	Hexane : isoamylalcohol (98 :2, v/v) (5 ml)	Sodium hydroxide (2M, 300-500 µl)	Sulphuric acid (0.025 M, 120 µl)	Direct injection
Hariharan *et al*. (1989)	Pentane : isopropanol (95:5, v/v) (6 ml)	Sodium hydroxide (14.8 mol/l, 5 drop)	Perchloric acid (100 mM, 2ml)	Mobile phase (100µ)
Midha *et al*. (1988)	Isopropanol : n pentane (5 :95, v/v) (10 ml)	Saturated sodium carbonate (0.5ml)	Hydrochloric acid (0.1 N, 1ml)	Acetonitrile (40µ)

Table 1.2 continued

References	Extraction solvent	Basifying agent	Back extraction solvent	Reconstitute solvent
Miller & Devane. (1986)	Hexane : isoamylalcohol (98 :2, v/v) (6 ml)	Sodium hydroxide (5 M, 100 µl)	Hydrochloric acid (0.1 M, 100µl)	Direct inject
McBurney & George. (1984)	Heptane : isoamylalcohol (98.5 :1.5, v/v)	Sodium hydroxide (2 M, 1 ml)	Sulphuric acid (0.005 M, 2ml)	Methanol
Dhar & Kutt. (1984)	Heptane : isoamylalcohol (15% in 1L) (5 ml)	Sodium hydroxide (1 mol/l, 0.5 ml)	Sulphuric acid (5 mM, 0.6ml)	Direct injection
Jatlow *et al.* (1983)	Hexane : isoamylalcohol (98 :2, v/v) (10 ml)	Sodium hydroxide (2 M, 0.2 ml)	Hydrochloric acid (0.1 M, 150µl)	Direct injection
Miyazaki & Arita. (1981)	Diethylether (4 ml)	Sodium hydroxide (1 N, 1 ml)	Hydrochloric acid (0.1 N, 3.5ml)	Direct injection
Bianchetti & Morselli. (1978)	Diethyleter (5 ml)	Sodium hydroxide (2 N, 200 µl)	Hydrochloric acid (0.2 N, 2.5ml)	
Hempenius *et al*. (1999)	Solid phase extraction			
Hoffman & Edkinds. (1994)	Solid phase extraction			
Cahard *et al.* (1990)	Solid phase extraction			

Abbreviation:
ml= mililiter; µl=microliter; M=molarity; N=normality; l=liter; mol/l=mol per liter; v/v=volume by volume; mM=milimolar

1.3.3 PHARMACOKINETICS OF HP AND RH

Numerous articles related to the absorption, distribution, metabolism and excretion of HP in animal (Braun *et al*., 1967; Soujin *et al*., 1967) and human (Cressman *et al*., 1974) have been reported. In most of these studies, pharmacokinetic characteristics of HP are usually reported after a single oral and intramuscular (IM) (Cressman *et al*., 1974) or intravenous (IV) administration in healthy volunteers (Holley *et al*., 1983; Chakraborty *et al*., 1989) and in schizophrenic patients (Cheng *et al*., 1987). A summary of the HP and RH pharmacokinetic parameters in animal and human are shown in Table 1.3.

HP is a lipophillic compound and therefore is rapidly absorbed from the gastrointestinal tract following oral administration, and undergoes a first pass metabolism in the liver. Due to the first-pass metabolism in the liver, plasma levels of HP occurs within 2 to 6 h after oral dosing (Kunka & Perel, 1989) and about 20 min after IM administration (Cressman *et al*., 1974) and the systemic bioavailability is approximately 75% (Adam & Fernandez 1987; Javaid, 1994). Oral bioavailability is about 65% after first pass metabolism (Holley *et al*., 1983).

HP is bound to plasma protein and the free fraction in serum is reported to be about 8 %(Forsman & Ohman, 1977a). It is widely distributed in the body and crosses the blood brain barrier and is also distributed in breast milk.

Pharmacokinetic studies of RH in normal volunteers (Chakraborthy *et al*., 1989; Midha *et al*., 1989) and schizophrenic patients (Chang *et al*., 1989a) have been reported. In a study by Chang *et al* (1989a), a single 10 mg dose of reduced haloperidol was given orally to seven male schizophrenic patients (age 43.4± 8.6 years; weight 54.6 ± 9.0 kg). The pharmacokinetic parameter of RH is shown in Table 1.3.

Table 1.3 Summary of pharmacokinetic for HP and RH

References	Model	Route	No	Dose (mg)	$t_{1/2}$ (h)	Vd (l/kg)	CL (l/h)	AUC (ng.h/ml)
	Haloperidol							
Cheng *et al*. (1987)	Human (67 ± 16kg)	Intravenous Oral	6 8	1.5 - 5.0 2.0 - 5.0	18.8 ± 4.7 18.1 ± 4.5	9.5 ± 1.9	33 ± 7.8	NR
Holley *et al*. (1983)	Human (70.9 ± 7.0kg)	Intravenous Oral	6 8	0.125 0.503	26.2 ± 8.0 14.5 ± 3.2	21.7 ± 6.9	49.2 ± 12	NR
Magliozzi & Hollister. (1985)	Human	Intravenous Oral	6 6	0.125 0.500	15.1 ± 2.5 17.5 ± 8.7	NR	NR	NR
Forsman *et al*. (1974)	Human	Intravenous	3	5	12.6	27	NR	NR
Cheng & Paalzow. (1992)	Rats (190 - 250g)	Intravenous	6 - 8	0.5 1.0 2.5	1.53 1.54 1.54	4.3 5.6 6.5	86.2[a] 82.3[a] 80.7[a]	5724.5 ± 4.6 11874.2 ± 3.0 32110.4 ± 2.9
Wurzburger *et al*. (1981)	Rats (300 - 350g)	Intravenous		0.25 0.5 1.0	2.69 2.60 2.50	3.33 4.60 4.50	53.2[b] 46.7[b] 42.2[b]	80.1 174 378
	Reduced haloperidol							
Chang *et al*. (1989a)	Human (54.6 ± 9.0 kg)	Oral	7	10	67.0 ± 51.3	46.0 ± 20.2	0.60 ±0.30[c]	448.5 ± 336.0[d]

Note:

a = Clearance reported at unit ml min^{-1} kg^{-1} ; b = Clearance reported at unit ml/min

c = Clearance reported at unit l/h/kg and d = AUC reported at unit µg.l/h

Abbreviations:

$t_{1/2}$ = elimination half-life; Vd=apparent volume of distribution; CL=clearance; NR=not reported

Table 1.4 Concentration of HP and RH in rat tissues after a single intraperitoneally injection of HP or RH (1mg/kg body weight) (Korpi & Wyatt, 1984).

Tissue/time after injection	HP treatment		RH treatment	
	HP	RH	HP	RH
Plasma				
10 min	0.2 ± 0.1	n.d.	0.0 ± 0.0	0.1 ± 0.1
30 min	0.1 ± 0.0	n.d.	0.0 ± 0.0	0.1 ± 0.0
120 min	0.1 ± 0.0	n.d.	0.0 ± 0.0	0.0 ± 0.0
Liver				
10 min	24.0 ± 7.8	0.4 ± 0.2	6.8 ± 0.5	1.8 ± 0.1
30 min	6.2 ± 0.2	0.1 ± 0.0	3.1 ± 0.1	0.8 ± 0.1
120 min	2.9 ± 0.2	0.0 ± 0.0	2.1 ± 0.1	0.3 ± 0.0
Corpus striatum				
10 min	3.4 ± 1.3	n.d.	0.2 ± 0.0	1.6 ± 1.3
30 min	3.2 ± 0.5	n.d.	0.5 ± 0.1	2.3 ± 0.9
120 min	1.2 ± 0.2	n.d.	0.6 ± 0.1	0.6 ± 0.2

Drug concentrations are given as mean ± S.E.M. (n=4) in µmol/l for plasma and in µmol/kg wet weight for liver and corpus striatum.
n.d.= not detected.

Table 1.5 Concentration of HP and RH in striatum and plasma after repeated IP injections of HP(Chang *et al*., 1988).

Dose (mg/kg)	Drug concentration			
	Striatum (ng/g)		Plasma (ng/ml)	
	HP	RH	HP	RH
0.1	138 ± 5	168 ± 10	1.8 ± 0.2	5.0 ± 0.4
0.5	220 ± 11	433 ± 16	4.4 ± 0.4	28.8 ± 2.0
2	436 ± 17	2158 ± 155	11.1 ± 0.6	149.6 ± 6.0

All values are mean ± S.E.M. of six to eight animals

Table 1.6 Pharmacokinetic parameters after administration of HP and RH.

References	Subject	No	Drug	Dose	pharmacokinetic parameters		
					AUC (ng.h/ml)	CL (l/kg)	$t_{1/2}$ (h)
Chakraborty *et al*., 1989	Healthy volunteers 77.6 ± 10kg	15	HP	(given HP 5 mg)	70.7 ± 33.4	NR	53.9 ± 24.5
			RH	(given HP 5 mg)	62.0 ± 103.1	NR	69.2 ± 23.9
			HP	(given RH 5 mg)	[a]	NR	[a]
			RH	(given RH 5 mg)	54.5 ± 20.9	NR	73.2 ± 29.4
Jann *et al*., 1990	Schizophrenic 43 - 66 kg	6	HP	(given HP 10 mg)	200.5 ± 99.2[b]	1.1 ± 0.3[c]	21.5 ± 9.1
			RH	(given HP 10 mg)	85.0 ± 72.2[b]	6.2 ± 8.8[c]	34.0 ± 22.5
			HP	(given RH 10 mg)	48.2 ± 66.4[b]	11.5 ± 10.9[c]	36.7 ± 48.0
			RH	(given RH 10 mg)	432.5 ± 336.3[b]	0.7 ± 0.6[c]	71.9 ± 61.2

Note:
a= could not be calculated in 14 out of 15 volunteers because HP not determined given RH dose. b= AUC reported at unit mcg.h/l.
CL = Clearance reported as unit l/h/kg.
Abbreviation : AUC = Area under the curve; HP = Haloperidol; RH = Reduced haloperidol; kg=kilogram; mg=miligram.

The liver is the major site of HP metabolism (Korpi *et al*., 1985). The metabolism of the HP in humans involves the initial cleavage of the molecule at the C-N bond of the central chain to form an inactive piperidine and 4-fluorobenzoylpropionic acid metabolites (Forsman *et al*., 1977) (Figure 1.3) and the formation of a conjugate with glucuronic acid at the hydroxy group (Oida *et al*., 1989). HP is also metabolised through reduction at the benzylic ketone group to form an alcohol metabolite, known as reduced haloperidol (RH) (Forsman & Larsson, 1978).

HP is excreted slowly in the urine and faeces. About 30% of a given dose is excreted in urine and about 20% of a given dose in faeces via biliary elimination (Beresford & Ward, 1987). Only 1% of a given dose is excreted as unchanged drug in the urine (Forsman *et al*., 1977). There is also evidence of enterohepatic recycling (Chakraborty *et al*., 1989).

The interconversion between HP and RH are species specific. RH is oxidised back to HP in rats (Table 1.4) (Korpi & Wyatt 1984; Korpi *et al*., 1985a), while in guinea pigs reversible metabolism of HP and RH (reduction and oxidation of both compounds) were found (Table 1.5) (Korpi *et al*., 1985, 1985a; Chang *et al*., 1988, 1991). The presence of RH in the plasma patients treated with HP was first reported by Forsman & Larsson (1978). However, the back conversion from RH to HP in human was not elucidated until 1987. Back conversion of HP to RH in humans was detected in plasma from healthy volunteer subjects. Healthy volunteer subjects were administration of a single oral 5 mg dose of RH (Midha *et al*., 1987). Two years later, these authors reported the interconversion between HP and RH in 14 of 15 normal volunteers who received a single 5 mg dose of HP and RH separately (Chakraborty *et al*., 1989; Midha *et al*., 1989). Further, Chang *et al*. (1989a) also reported the reversible metabolism of HP and RH in schizophrenic patients (Table 1.6). The enzyme

responsible to produce RH from HP is characteristic of a ketone reductase that is present in human and guinea pig hepatic cytosol (Inaba & Kovac, 1989). The reductase activity was dependent on nicotinamide-adenine-dinucleotide-phosphate as a cofactor for both humans and guinea pigs.

Several metabolic pathways involved in the metabolism of HP and RH are mediated by cytochrome P_{450} isoenzymes (CYP) (Usuki *et al*., 1998). CYP3A4 plays an important role and is responsible for the N-dealkylation of HP and RH (Pan *et al*., 1997; Pan *et al*., 1998a; Fang *et al*., 1997), the back oxidation of HP to RH (Pan *et al*., 1998a, Kudo & Odomi, 1998; Avent & Gillam, 1998; Fang *et al*., 1997) and the formation of pyridinum metabolite (Avent & Gillam, 1998; Fang *et al*., 1997; Eyles *et al*., 1996). There are however, in the literature, several suggestions based on *in vivo* and *in vitro* studies, that CYP2D6 (Inaba *et al*., 1985; Tyndale *et al*., 1991) could also be involved, but the metabolic pathways are not known.

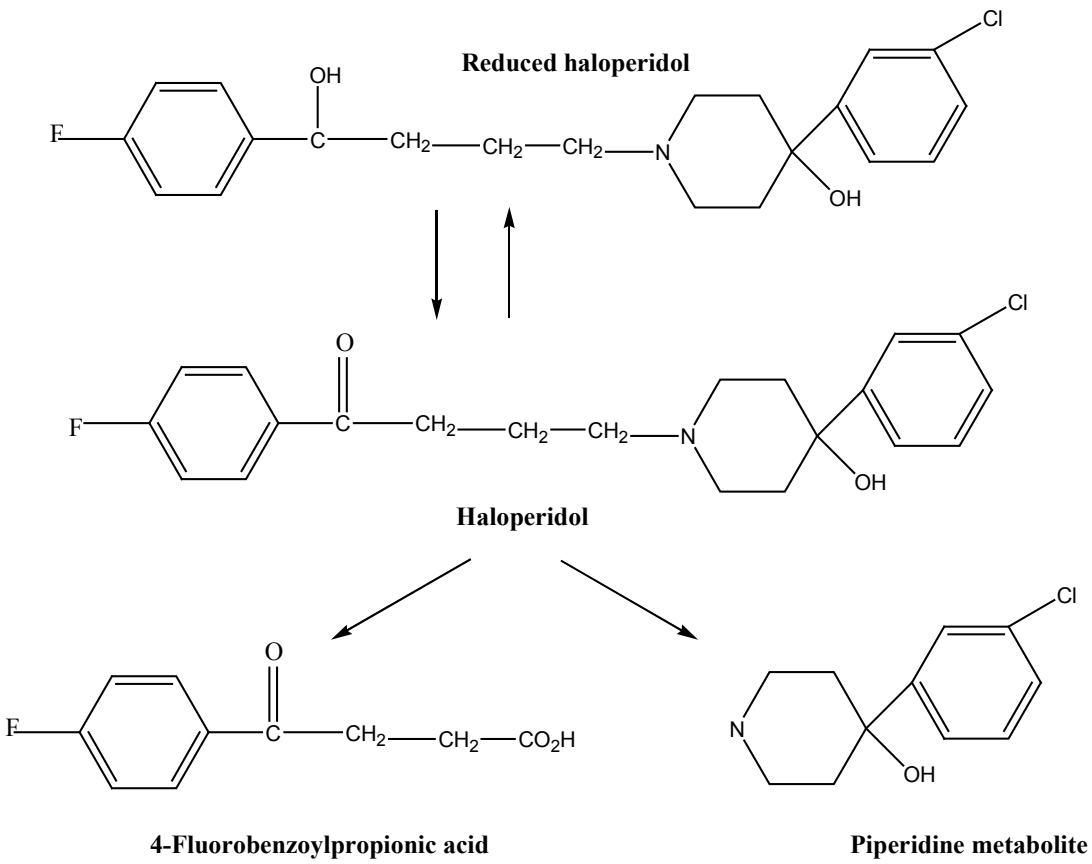


Figure 1.3 Metabolism of HP (Forsman e*t al*., 1977).

1.3.4 DRUG INTERACTIONS

A drug interaction usually refers to the modifications of the expected drug response due to exposure of the patients to other foods or drugs administered concomitantly. Drug interaction may include drug-drug interaction or drug-food interaction (Shargel & Yu, 1999). Food-drug interactions can produce negative effects in the safety and efficacy of drug therapy and in the nutritional status of the patients (Miguel *et al*., 2005). Many nutrients substantially interfere with the absorption or metabolism of drugs in the body (Anderson, 1998; Kirk, 1995). For examples, grapes fruit juice increase the plasma level of many drugs such as lovastatin and simvastatin due to naringin that inhibit their metabolism (Kantola *et al*., 1998). Fluoroquinolones binds with iron or calcium enriched foods and antacids if administered simultaneously. The resulting compounds excreted with little or no systemic absorption. Significant interaction of P-glycoproteins with cyclosporine and reduce the absorption of levofloxacin has been reported (Wallace *et al*., 2003).

Many drug-drug interactions are metabolism based and related to cytochrome P_{450} (CYP) enzymes. The CYP enzyme system is a very large group of enzymes encoded by the P_{450} superfamily. CYPs are membrane bound proteins with an approximate molecular weight of 50 kD and contain a heme moiety (Gunaratna, 2000). Because of the diversity of the cytochrome family a nomenclature system based on sequence identity is developed. This nomenclature is comprised of family and sub-family systems. Family includes CYPs and is designated by a number after CYP. Sub-family is the CYPs within the family and is designated by a letter following the number. For example, CYP2D6 is a cytochrome P_{450} enzyme. It belongs to family 2 and sub-family D. The last number 6 refers to the sequence of discovery. There are about 30 human cytochrome P_{450} enzymes. Only 6 of them such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are mainly involved in drug metabolism.

Among these CYP3A4 is the most abundant and most clinically important isoenzymes in humans (Gunaratna, 2000).

The enzymes involved in the metabolism of drug are altered by food and the co-administered drug. Enzyme induction is a drug or chemical-stimulated increase in enzyme activity usually due to an increase in the amount of enzyme present. Enzyme inhibition is substrate competition or due to direct inhibition of the drug metabolising enzymes, mainly CYP enzymes (Shargel & Yu, 1999). The CYP enzyme is important to the pharmacokinetics of psychotropic drugs. The result of inhibition is a higher plasma level of drug that can cause adverse effect. The result of induction is a lower plasma level of drug that can affect the therapeutic efficacy (Sharif, 2003).

Concomitantly administered drugs may affect the pharmacokinetics of HP by influencing its metabolic clearance and its ability to bind to plasma proteins, and by causing alterations in hepatic blood flow. HP appears to be a moderately extracted drug. On a theoretical basis, alterations in protein binding of drugs with a low extraction ratio would have no influence on the pharmacokinetics of unbound drug at steady state. On the other hand, alterations of plasma protein binding of high extraction drugs would be predicted to cause a change in the clearance of unbound drug (Wilkinson & Shand, 1975; Nies *et al*., 1976; Blaschke, 1977).

Table 1.7 shows pharmacokinetic interactions between HP and other drugs. Anticonvulsant drugs such as carbamazepine, phenytoin and phenobarbitone are classified as enzyme inducers. Clinically significant decrease in plasma concentration of HP was reported when coadministered with carbamazepine (Jann *et al*., 1985; Kidron, *et al*., 1985; Arana *et al*., 1986). Another similar study showed decreased plasma concentration of HP when co-administered with phenytoin and phenobarbitat (Linnoila *et al*., 1980). There have been several reports on