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Method Development and Validation of the Quantitation of 19 Antipsychotics Using Deuterated Internal Standards

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Method Development and Validation of the Quantitation of 19 Antipsychotics using Deuterated Internal Standards

by

Anthony Shawn Epps

An Abstract of a Thesis
in
Forensic Science

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

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State University of New York
College at Buffalo
Department of Chemistry

ABSTRACT OF THESIS

Method Development and Validation of the Quantitation of 19 Antipsychotics using Deuterated Internal Standards

Antipsychotic drugs or neuroleptics are used primarily for psychiatric disorders such as schizophrenia, psychosis, and bipolar disorder. In forensic science antipsychotics are drugs of considerable interest because of their potential abuse, involvement in suicides, and they are frequently associated with sudden death investigations. Well-characterized and fully validated analytical data is necessary to generate reproducible and reliable results. As a result, data can be correctly interpreted and objectively demonstrate in its applicability for the intended use. This research has developed and validated a method that is selective, sensitive and accurate using liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the simultaneous determination of approximately 19 antipsychotics with deuterated internal standard in postmortem human blood. Compound optimization parameters for detecting a specific compound as well as a quantitative assay of the antipsychotic compounds were accomplished. Validation parameters such as determination of linearity, within and between day accuracy and precision, carryover evaluation, matrix effect and recovery, and peak purity and selectivity study were performed. These parameters yielded results for all of the compounds with only two (mesoridazine and thiothixene) requiring further study to improve their performance. The validated method has been successfully used to analyze postmortem human blood for application in forensic toxicology.

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instrumentation. As a result, research life became smooth and rewarding for me and this success is as much his as it is mine.

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List of Abbreviations

Δ P – Pressure difference
μ – The symbol for micro
A/S – Autosampler
AA – Atomic Absorption Spectroscopy
AC/DC – Alternating Current or Direct Current
ACN – Acetonitrile
AMU – Atomic mass unit
AP MALDI – Atmospheric pressure matrix-assisted laser desorption/ionization
APCI – Atmospheric pressure chemical ionization
API – Atmospheric pressure ionization
As – Asymmetry
Atm – Atmospheres
AU – Absorption Units
BP – Base peak
CA – Collisional activation
CAD – Collisionally activated dissociation
CE – Capillary Electrophoresis
CE – collision energy
CEP (CCEP) – collision cell entrance potential
CES – collision energy scan (collective scan of 20, 35, and 50 CEs)
CI – Chemical ionization
CID – Collision-induced dissociation
CN – Cyano
CRM – Consecutive reaction monitoring
CXP – collision cell exit potential
DC – Direct Current
DP – declustering potential
DI – Deionized, as in water
DESI – Desorption electrospray ionization
EA – Electron affinity
ECD – Electron-capture dissociation
EI – Electron ionization (or electron impact)
EP – entrance potential
EPI – enhanced product ion scan (anything with enhanced = ion trap = Q3)
ESI – Electrospray Ionisation
EtOH – Ethanol

eV – Electronvolt
FD – Field desorption
FTMS – Fourier transform mass spectrometer
GC – Gas Chromatography
GC-MS – Gas Chromatography - Mass Spectrometry
HAc – Acetic Acid
He - Helium
HPLC – High Performance Liquid Chromatography
i.d. – Internal diameter
IC – Ion Chromatography
IEX – Ion Exchange Chromatography
IPA – Isopropyl Alcohol
IPLC – Ion Pair Liquid Chromatography or RP-HPLC with an ion pair reagent in the eluent.
IS – Internal Standard
IT – Ion trap
K – Potassium
K – Distribution Constant or Kelvin (Absolute temperature)
k' – Relative Retention time
LC/MS/MS – Liquid Chromatography with Tandem Mass Spectrometry
LC-MS – Liquid Chromatography with Mass Spectrometry
LIT – Linear ion trap
LOD – Limit of Detection.
LOQ – Limit of Quantitation.
m/z – Mass to charge ratio
MCA – averaging mechanism for the scans
MeOH – Methanol
MRM – multiple reaction monograph
MS – Mass spectrometer
MS – Mass spectrometry
MS/MS – Mass spectrometry/mass spectrometry, i.e. tandem mass spectrometry
MRM – Multiple reaction monitoring
MW – Molecular Weight
Mz – Average molecular weight
N – Efficiency (from Number of theoretical plates).
Na – Sodium
NH_2 – Amino
NH_4 – Ammonium
NMR – Nuclear Magnetic Resonance
o.d. – Outer diameter
Pa – Pascal (unit of pressure)
pH – A measure of acidity
Ph – Phenyl
ppm, ppb, ppt – parts per million, parts per billion, parts per trillion.
psi – Pounds per square inch

PT – Peak Threshold
PW – Peak Width
QA – Quality Assurance
QIT – Quadrupole ion trap
QMS – Quadrupole mass spectrometer
Quad – Quadrupole
QUISTOR – Quadrupole ion storage trap
R^2 – Coefficient of determination
RF – Response factor
RF – Radio Frequency
RP HPLC – Reversed Phase High Performance Liquid Chromatography
RPLC – Reversed Phase Liquid Chromatography
Rs – Resolution
S/N ratio – Signal to Noise Ratio
SAX – Strong Anion Exchanger
SCX – Strong Cation Exchanger
SEC – Size Exclusion Chromatography
SEM – Scanning Electron Microscopy
SI units – The International System of Units
Sil – Silica
SIM – Single Ion Monitoring
SIM – Selected ion monitoring
SIR – Selected ion recording
SID – Surface-induced dissociation
SIR – Surface-induced reaction
SI – Surface ionization
SOP – Standard Operating Procedure
SPE – Solid Phase Extraction
SRM – Selected reaction monitoring
THF – Tetrahydrofuran
TI – Thermal ionization
TIAFT – The International Association of Forensic Toxicologists
TIC – Total Ion Count
TIC – Total ion chromatograph
TIC – Total ion current
TICC – Total ion current chromatogram
TLC – Thin Layer Chromatography
TMT – Tandem mass tags
TMS –Trimethylsilyl
TMS –Trimethylsilane
TOF – Time of Flight
TOF-MS – Time-of-flight mass spectrometer
tr – Retention time
UPLC – Ultra Performance Liquid Chromatography
UV – Ultraviolet

UV-VIS – The Ultraviolet Visible spectrum
v/v or w/v – Volume for volume or weight to volume
Vol % – Volume percent
WAX – Weak Anion Exchanger
WCX – Weak Cation Exchanger

CHAPTER ONE: Introduction

1.1 Antipsychotic

Antipsychotic drugs or neuroleptics are used primarily for psychiatric disorders such as schizophrenia, psychosis, and bipolar disorder. They have also been used for non-psychotic disorders such as depression, behavioral disorders, mood stabilizers, hypnotic (sleep) medications and many other off-label uses [1]. Toxicity varies considerably with each drug however the general effects are similar including sedation, hypotension, tachycardia, dysrhythmias and seizures. Most antipsychotics are divided into two groups; the typical or first-generation antipsychotics, and the atypical or second-generation antipsychotics.

A first generation of antipsychotics are known as typical antipsychotics, and were discovered in the 1950s when chlorpromazine, which was developed as a surgical anesthetic, was used on psychiatric patients because of its sedative and powerful calming effect. Because of this tranquilizing effect, typical antipsychotics have also been referred to as the major tranquilizers [2]. Regular and high levels of sedation are common among this group of antipsychotics and when used heavily they can be incapacitating. It is believed that by blocking dopamine receptors from excess dopamine released in the brain, psychotic experiences can be controlled. Typical antipsychotics block the

dopamine D_2 receptor, but are very unselective blocking "D2-like family" receptors in the mesocortical pathway, tuberoinfundibular pathway and the nigrostriatal pathway. This lack of selectivity is believed to be responsible for many of the side-effects caused by these drugs. These unwanted side effects such as extrapyramidal motor control disabilities, include problems like acute dystonias, akathisia, parkinsonism, tardive dyskinesia, tachycardia, hypotension, seizures, hyperprolactinaemia, and many others that can become permanent [3] [4]. Typical antipsychotics are classified based on their potency which is a measure of the relative effectiveness of antipsychotics compared to chlorpromazine [5]. High-potency antipsychotics have a high affinity for the D_2 receptors and are more likely to cause adverse effects (extrapyramidal effects) associated with these receptors. However, there are fewer adverse effects (sedation) caused by other receptors due to the increased selectivity for the D_2 receptors. Low-potency antipsychotics have low selectivity of the D_2 receptors lowering symptoms such as extrapyramidal effects but increasing those such as sedation [6]. Potency, however, does not equate to efficacy because despite differences in structure and D_2 receptor affinity, all typical antipsychotics have clinically similar efficacy at their standard dose. Because of the chronic nature of the treated disorders, antipsychotic medications are rarely discontinued once started, potentially causing permanent chemical dependence leading to a much worse psychosis [7]. They are usually only stopped if the side-effects become too severe or a patient has been symptom free for a long period of time. Discontinuation of typical antipsychotics requires critical managing, consistent treatment and a good understanding of the withdrawal symptoms (nausea, emesis, anorexia, diarrhea,

rhinorrhea, diaphoresis, myalgia, paresthesia, anxiety, agitation, restlessness, and insomnia), some of which may be permanent, such as Tardive dyskinesia.

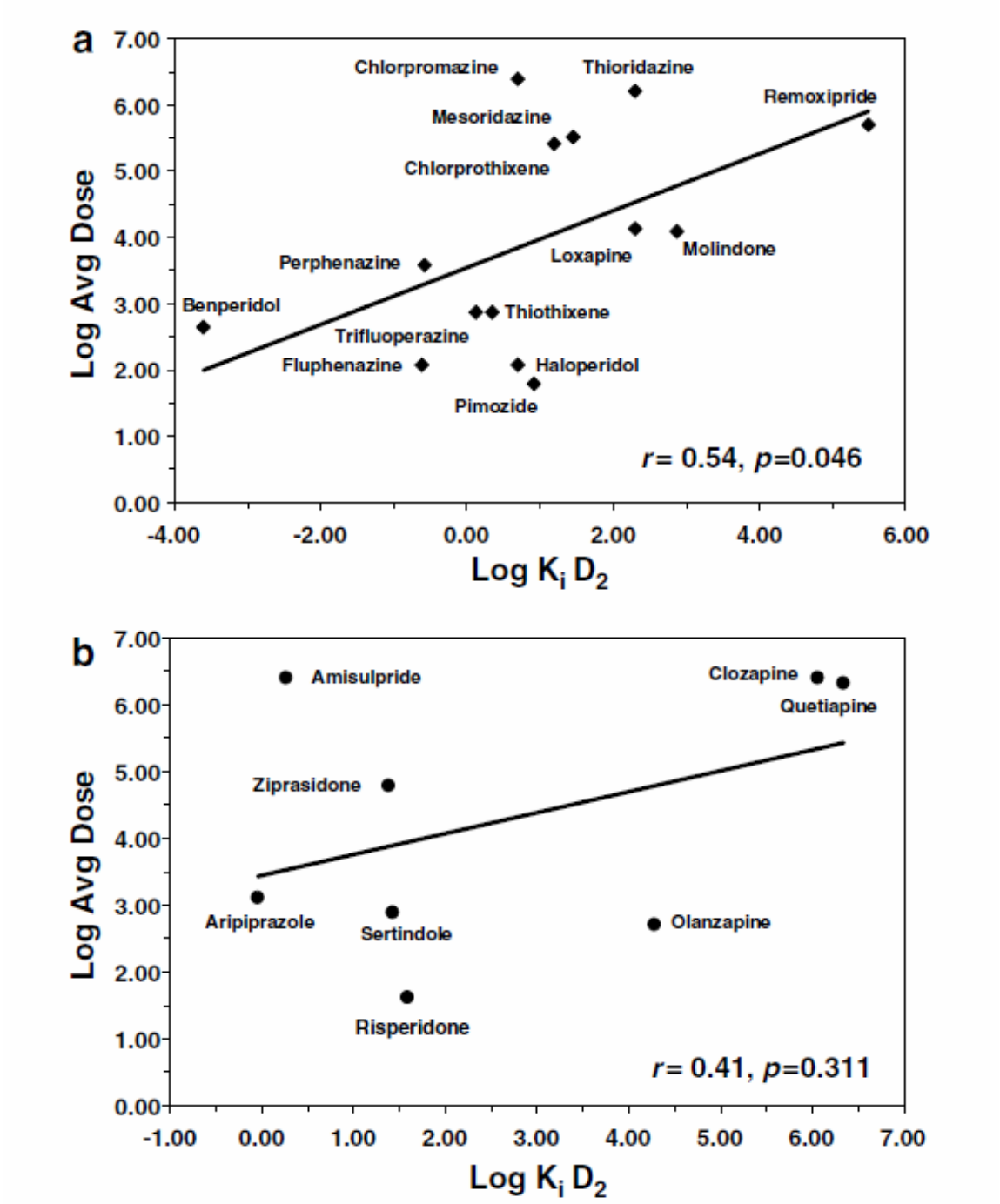


Figure 1: Clinically effective antipsychotic dose vs. binding affinity to cloned human DA D_2 receptor for (a) typical and (b) atypical antipsychotic medications (NM Richtand et al).

The first atypical antipsychotic, clozapine, was discovered in the 1950s and introduced clinically in the 1970s but many others have been developed more recently. Second generation (atypical) antipsychotics are potent serotonin antagonists, readily bind with D3 and D4 dopamine receptors (restricted to the neurons of the limbic system and cerebral cortex) and act considerably less on D_2 receptors reducing the adverse effects. Unlike typical antipsychotics, the affinity for D_2 receptors does not correlate with their clinically effective dose and important differences between typical and atypical antipsychotics are the side effects (Figure 1). Atypical antipsychotics have a greater efficacy than that of typical antipsychotics for the treatment of psychosis and are easier to discontinue being less likely to cause physical dependency and withdrawal symptoms. However, atypical antipsychotics are excreted faster than the typical antipsychotics so relapses into psychosis are greater. There are still a number of harmful and adverse effects that vary among these different antipsychotics such as lowered life expectancy, weight gain, decrease in brain volume, agranulocytosis, diabetes, sexual dysfunction, tardive psychosis, and tardive dysphrenia [8] [9].

In 2008, antipsychotics were among the leading class of prescribed drugs in the US and had a global value of about $22 billion [8]. Increasing off-label (non-FDA approved) uses for atypical antipsychotics are targeting an increasing array of psychiatric indications such as dementia in older people, attention-deficit/hyperactivity disorder, conduct disorder, and affective disorders in children and adolescents [11] and for the treatment of irritability in children and adolescents with autism [12]. Unfortunately, the side effects of using antipsychotics for off-label usages are unknown leading to the deaths

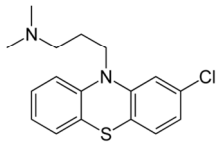
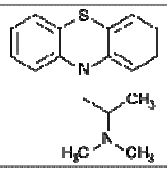
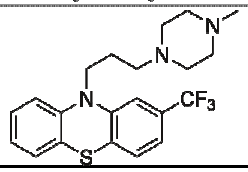
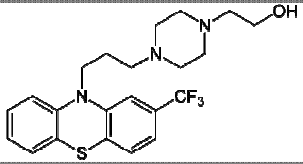
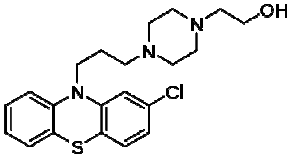
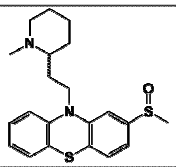
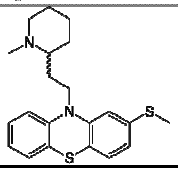
of patients taking the drugs. Use of these drugs continues to increase despite studies indicating that adverse side-effects are more severe in children than adults [13] [14] [15]. These drugs can increase the risk of sudden cardiac death, with mortality rates increasing with age and dosage [16] [17]. Although atypical antipsychotic drugs have a favorable adverse effect profile relative to traditional antipsychotics, there is still much needed study into the severity of their side-effects. Many of these drugs carry warnings stating elderly patients with dementia-related psychosis treated with antipsychotic drugs are at an increased risk of death. The risk of death is increased when these drugs are combined with alcohol, certain food items or other medications. Toxicity among antipsychotic drugs is extremely variable with patients surviving massive overdoses and others dying as a result of taking therapeutic doses. This variability may result from the fact that the metabolic pathways and multiple drug interactions of antipsychotics are not completely understood [18].

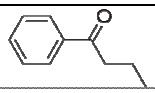
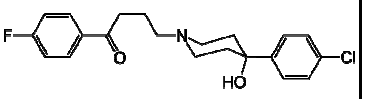
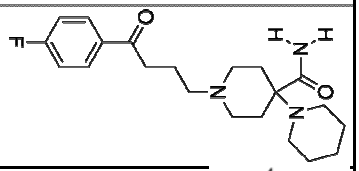
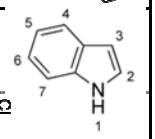
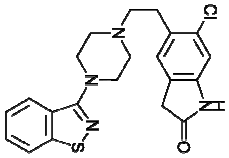
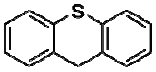
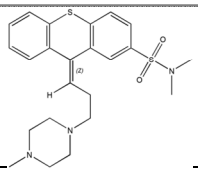
In forensic science, the antipsychotics are drugs of considerable interest because of their potential abuse and their involvement in suicides and intoxication. Many deaths caused by atypical antipsychotic toxicity are often related to cardiovascular complications, but pulmonary, neurological, endocrine and gastrointestinal complications have also caused fatalities. This can result in misdiagnosing the actual cause of death unless antipsychotic drugs are tested for and the role they play in certain deaths is understood. This requires that an assay to identify and quantitate these drugs from sub to supratherapeutic doses be developed and validated.

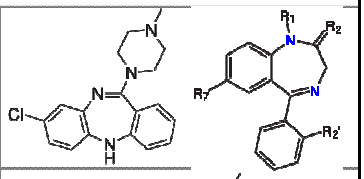
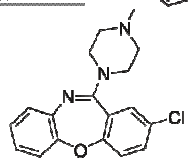
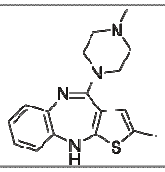
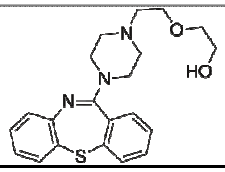
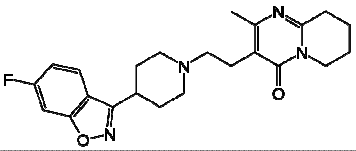
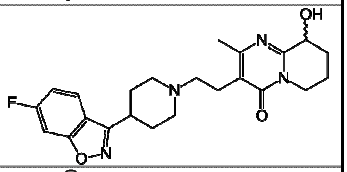
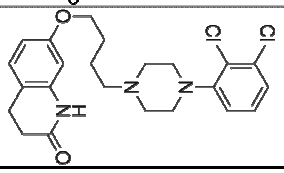
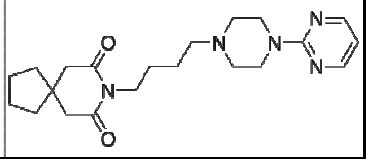
1.2 Antipsychotics in this Study

In an effort to validate a method to identify and quantitate antipsychotics in postmortem human blood, 19 antipsychotics were chosen. The choice of which drugs to use was based on prescribed antipsychotics in case files found at the scene in the last two years. Also considered were drugs commonly used in Western New York and already screened for by the Erie County Toxicology Lab. Table 1 contains these drugs and the various structures, categories and classes they belong to and Table 2 contains drug forms, dosage, therapeutic blood levels and other drug information. Lastly Table 3 contains the TIAFT reference blood level list of these drugs with their therapeutic and toxic amounts.

Table 1: Antipsychotics in this study, grouped by chemical class, type, potency for typical, and structure.

Aliphatic Phenothiazines			
Chlorpromazine	Low Potency	Typical	
Promethazine	Low Potency	Typical	
Trifluoperazine	Medium Potency	Typical	
Piperazine Phenothiazines			
Fluphenazine	High Potency	Typical	
Perphenazine	Medium Potency	Typical	
Piperidine Phenothiazines			
Mesoridazine	Low Potency	Typical	
Thioridazine	Low Potency	Typical	

Butyrophenone 			
Haloperidol	High Potency	Typical	
Pipamperone	Low Potency	Typical	
Indoles 			
Ziprasidone	Atypical		
Thioxanthines 			
Thiothixene	Medium Potency	Typical	

Benzodiazepines			
Clozapine	Atypical		
Loxapine	Medium Potency	Typical	
Olanzapine	Atypical		
Quetiapine	Atypical		
Other			
Risperidone	Atypical		
Paliperidone (9-Hydroxyrisperidone)	Atypical		
Aripiprazole	Atypical		
Buspirone	Generalized Anxiety Disorder		

1.2.1 9-hydroxyrisperidone

Paliperidone (Brand names: INVEGA and INVEGA Sustenna), also known as 9-hydroxyrisperidone, is an atypical antipsychotic developed by Janssen Pharmaceutica. Racemic 9-hydroxyrisperidone is itself licensed for the management of schizophrenia (Paliperidone®). It was approved by the US Food and Drug Administration (FDA) for schizophrenia in 2006 (Invega), 2009 (Invega Sustenna). Invega is an extended release formulation for once-daily dosing and Invega Sustenna (paliperidone palmitate) is a long-acting injectable formulation for once-monthly dosing. 9-hydroxyrisperidone is an active metabolite of the atypical antipsychotic risperidone by hydroxylation and N-demethylation. There are only a few reported cases of paliperidone overdose, with the highest amount ingested being 405 mg (Invega package insert). Observed signs and symptoms included extrapyramidal symptoms and gait unsteadiness. Since 9-hydroxyrisperidone is an active metabolite, overdoses reported with risperidone will be similar.

1.2.2 Aripiprazole

Aripiprazole (Brand names: Abilify, Abitat, Aripiprex) is an atypical antipsychotic antidepressant used in the treatment of schizophrenia, bipolar disorder, and clinical depression. Aripiprazole is a dopamine D2-receptor antagonist that has been used since 1999. It was approved by the US Food and Drug Administration (FDA) for schizophrenia (2002), bipolar disorder (2004), depressive disorder (2007), and irritability

in children with autism (2009). Off-label uses consist of the treatment of behavioral problems in elderly people with dementia and the treatment of other behavior problems. Overdose symptoms may result in drowsiness, vomiting, confusion, seizure, or coma however no fatalities have been reported.

1.2.3 Buspirone

Buspirone (Brand name: Buspar) is a psychoactive drug and pharmaceutical medication used primarily as an anxiolytic, specifically for generalized anxiety disorder. It is the first drug of this type in a chemical group known as the azaspirodecanediones, which are chemically unrelated to other existing anxiolytic or antipsychotic agents. Buspirone was approved by the US Food and Drug Administration (FDA) for generalized anxiety disorder (1986). Buspirone is sometimes used off-label for augmentation of selective serotonin reuptake inhibitor (SSRI) therapy for depression. An overdose with Buspirone can cause an increase in symptoms such nausea, vomiting, drowsiness, and stomach pain however no fatal cases have been reported [17].

1.2.4 Chlorpromazine

Chlorpromazine (Thorazine, Largactil) is a typical antipsychotic synthesized in 1952, and the first drug developed with specific antipsychotic action. Chlorpromazine is the first and most useful of the phenothiazine derivatives due to its ability to dramatically improve the prognosis of patients in psychiatric hospitals. Off-label uses for

chlorpromazine have been in the treatment of severe migraines, and in cancer patients to improve nausea and intensify and prolong the analgesic action of opioids given. Therapeutic doses of chlorpromazine are capable of causing severe adverse effects that can be potentially fatal. Sudden death ("phenothiazine sudden death") has also occurred with many psychiatric patients receiving large daily doses of chlorpromazine or other phenothiazines [17].

1.2.5 Clozapine

Clozapine (Brand name: Clozaril, Azaleptin, Leponex, Fazaclo, Froidir; Denzapine, Zaponex, Klozapol, Clopine). This was the first of the atypical antipsychotics to be developed, and it was first introduced in Europe in 1971, however it was voluntarily withdrawn by the manufacturer in 1975 after it was shown to cause agranulocytosis (dangerous decrease of white blood cells) that led to fatalities. Since Clozapine is more effective than any other antipsychotic for treating schizophrenia, it is usually used as a last resort in patients that have not responded to other anti-psychotic treatments. In 1989, the U.S. Food and Drug Administration (FDA) approved clozapine's use for treatment-resistant schizophrenia with required blood testing and in 2002 for reducing the risk of suicidal behavior for patients with schizophrenia. Clozapine has a black box warning for agranulocytosis, seizures, myocarditis, and adverse cardiovascular and respiratory effects. Off-label uses have included treatment of intractable chronic insomnia, schizoid personality disorder, resistant bipolar disorder, resistant paranoid personality disorder,

and resistant delusional disorder. In addition to the therapeutic hazards acute overdose can cause hypotension, cardiac arrhythmias, respiratory depression, coma and death [17].

1.2.6 Fluphenazine

Fluphenazine (Brand name: Permitil, Prolixin) belongs to the piperazine class of phenothiazines and is a typical antipsychotic drug. It was approved by the U.S. Food and Drug Administration (FDA) in 1968 for the treatment of psychoses such as schizophrenia and acute manic phases of bipolar disorder. Fluphenazine can cause irreversible side effects such as tardive dyskinesia and potentially fatal neuroleptic malignant syndrome. Some patients have been placed on very high doses of fluphenazine without any adverse effects (300-1200 mg per day [20]) however other have died at lower doses.

1.2.7 Haloperidol

Haloperidol (Brand names: Haldol, Serenace) a butyrophenone derivative is a typical antipsychotic used in the treatment of schizophrenia. It has pharmacological effects similar to the phenothiazines. Haloperidol was discovered by Paul Janssen and was developed in 1958 by Janssen Pharmaceutica. It was approved by the U.S. Food and Drug Administration (FDA) in 1967. Off-label uses for haloperidol have mainly been for its sedating effects. High-doses of haloperidol have resulted in bradycardia, hypothermia, sinus arrhythmia, neuromuscular rigidity, tremors, coma, and death [17].

1.2.8 Loxapine

Loxapine (Brand names: Daxoline, Loxapac, Loxitane) is a typical antipsychotic medication, approved by the U.S. Food and Drug Administration (FDA) in 1976 for the treatment of schizophrenia. Loxapine is a dibenzazepine derivative that is structurally similar to clozapine and behaves as an atypical antipsychotic [21]. High-doses of loxapine have caused dizziness, confusion, weakness, extrapy-ramidal effects, stupor, tachycardia, profound hypotension, respiratory depression, seizures, coma, and death [17].

1.2.9 Mesoridazine

Mesoridazine (Brand name: Serentil) is a side-chain sulfoxide of thioridazine belonging to the class of drugs called phenothiazines, used in the treatment of schizophrenia. It is a metabolite of thioridazine and was first synthesized in 1963 and has been in clinical use since 1965. Although it's pharmacological activity is similar to thioridazine, its effects have shown it to be more potent. Mesoridazine was withdrawn from the United States market in 2004 because it may cause life-threatening irregular heartbeats [20]. Blood concentrations of mesoridazine are similar in high-dose chronic therapy to those of fatal cases and deaths have been reported with ingestion of 2.5 to 10 g of the drug [17].

Table 2: Medical and chemical information about antipsychotics in this study (**[17]** and other sources)

Drug	Form	Type		Dose	Daily Dose Range	Plasma Concentration
9-Hyroxyrisperidone		Oral	Tablets		3 - 12 mg	
		Intramuscular	Injection		39 - 234 mg	
Aripiprazole	Free Base	Oral	Tablets	2 - 30 mg	2 - 30 mg	120-452 µg/L
			Syrup	1 mg/mL		
		Intramuscular	Injection	7.5 mg/mL		
Buspirone	Hydrochloride Salt	Oral	Tablets	5 - 10 mg	15 - 60 mg	0.21-3.07 µg/L
Chlorpromazine	Hydrochloride Salt	Oral	Tablets	10-300 mg	25 - 2400 mg	0.010-0.3 mg/L
		Rectal	Suppositories			
		Oral	Syrup	10 mg/5 mL		
			Concentrate	30-100 mg/mL		
		Intramuscular	Injection	25 mg/mL		
Clozapine	Free Base	Oral	Tablets	25 and 100 mg	12.5-400 mg	0.06-0.65 mg/L
Fluphenazine	Hydrochloride Salt	Oral	Tablets	1-10 mg	2.5-20 mg	0.26-22.6µg/L
			Syrup	2.5 mg/5 mL		
		Intramuscular	Injection	2.5 mg/mL		
	Decano/Enanthate Esters	Intramuscular	Injection	12.5-100 mg every 1-3 weeks.		
Haloperidol	Lactate Salt	Oral	Tablets	0.5-20 mg	0.5 - 100 mg	0.5-245 µg/L
			Concentrate	2 mg/mL		
		Parenteral	Injection	5 mg/mL		
		Intramuscular	Injection	50 - 100 mg/mL		
	Decanoate ester	Intramuscular	Injection	50-450 mg once monthly		
Loxapine	Succinate Salt	Oral	Capsules	5-50 mg	20-250 mg	0.002 - 0.030 mg/L
	Hydrochloride Salt		Concentrate	25 mg/mL		
		Intramuscular	Injection	50 mg/mL		
Mesoridazine	Besylate Salt	Intramuscular	Injection		50-400 mg	0.10 - 1.05 mg/L
		Oral	Tablets			
Olanzapine	Free Base	Oral	Tablets	2.5-10 mg	10-20 mg	9.3 - 26 µg/L
Perphenazine	Free Base	Intramuscular	Injection	5-10 mg		0.0003 - 0.03 mg/L
		Oral	Tablets	12-64 mg		
	Enanthate Ester	Intramuscular	Injection	100 mg every 14 days		
Promethazine	Hydrochloride Salt	Oral	Tablets	12.5-50 mg	25-150 mg	3-99 µg/L
			Syrup	6.25 mg/5 mL		
		Parenteral	Injection	25 and 50 mg/mL		
		Rectal	Suppositories	12.5-50 mg		
Quetiapine	Fumarate Salt	Oral	Tablets	25 - 200 mg	150-750 mg	0.14 - 0.402 mg/L
Risperidone	Free Base	Oral	Tablets	1-4 mg	2-6 mg	4-27 µg/L
Thioridazine	Hydrochloride Salt	Oral	Tablets	100-800 mg		0.05 - 3.9 mg/L
			Concentrate			
Thiothixene	Hydrochloride Salt	Intramuscular	Injection	20-60 mg		0.002 - 0.1 mg/L
Trifluoperazine	Hydrochloride Salt	Oral	Tablets	1-10 mg	2-40 mg	0.5-28 µg/L
			Concentrate	10 mg/mL		
		Intramuscular	Injection	2 mg/mL	6 mg	
Ziprasidone	Hydrochloride Salt	Oral	Tablets	5 - 20 mg	10-120 mg	45-139 µg/L
			Capsules			
		Parenteral	Injection	10 mg/mL		

1.2.10 Olanzapine

Olanzapine (Brand names: Zyprexa, Zalasta, Zolafren, Olzapin, Oferta, Zypadhera) is an atypical antipsychotic that is a serotonin and dopamine-receptor antagonist with anticholinergic properties. Olanzapine was approved by the U.S. Food and Drug Administration (FDA) in 1996 for the treatment of schizophrenia and bipolar disorder. Olanzapine is structurally similar to clozapine, and is classified as a benzodiazepine. Off-label uses of olanzapine have included generalized anxiety disorder, [23] panic disorder, [24] delusional parasitosis, [25] post-traumatic stress disorder, [26] anorexia nervosa, [27] Tourette syndrome [28] and stuttering [29]. Overdoses with 300 mg of olanzapine in an adult caused only drowsiness and slurred speech however death has been reported after an acute overdose of 600 mg, but others have survived after acute overdoses of 1000 mg or more [17].

1.2.11 Perphenazine

Perphenazine (Brand name: Trilafon) is a typical antipsychotic drug and a chlorpromazine analogue, classified as a piperazinyl phenothiazine. It was first synthesized in 1956 and approved by the U.S. Food and Drug Administration (FDA) that same year. High-dose therapy and overdoses of perphenazine can have numerous adverse reactions such as extrapyramidal effects, tardive dyskinesia, anticholinergic effects, drowsiness, postural hypotension, blood dyscrasias, obstructive jaundice and sudden death [17].

1.2.12 Pipamperone

Pipamperone (Brand name: Dipiperon) is an antipsychotic drug (a butyrophenone derivative) used in the treatment of schizophrenia [30]. Pipamperone is used for its sedative effects in chronic psychotic patients [31]. Pipamperone in supratherapeutic doses may lead to torsade de pointes, a life-threatening ventricular tachyarrhythmia [32].

1.2.13 Promethazine

Promethazine (Brand names: Phenergan, Remsed. Zipan, Promethegan, Romergan, Fargan, Farganesse, Prothiazine, Avomine, Atosil, Receptozine, Lergigan, and Sominex) is a typical antipsychotic. Promethazine is a phenothiazine derivative and a H1 receptor antagonist that since 1950 has been used as an antihistamine (allergic reactions & common cold), sedative and occasionally for insomnia. Promethazine is also used to prevent and treat motion sickness, nausea and vomiting or pain after surgery. It is available over the counter in many other countries and is not used as an antipsychotic having only a tenth of the antipsychotic strength of chlorpromazine. Adverse reactions to promethazine are similar to related antipsychotics however an overdose of promethazine can cause coma, convulsions, circulatory failure and respiratory depression which has caused death in a child younger than 2 (Promethazine package insert).

1.2.14 Quetiapine

Quetiapine (Brand names: Seroquel, Ketipinor), is an atypical antipsychotic and a dibenzothiazepine derivative developed in 1993 and FDA approved in 1997 for the treatment of schizophrenia, and mania-associated bipolar disorder. Quetiapine is structurally similar to clozapine, and is classified as a benzodiazepine but unlike clozapine it exhibits lesser affinity for dopaminergic, adrenergic and histamine receptors. Quetiapine has been used off-label to treat obsessive-compulsive disorder, post-traumatic stress disorder, autism, alcoholism [33], Borderline Personality Disorder [34], Tourette syndrome, [35] and for sleep disorders or anxiety disorders [36] [37]. Acute overdoses have resulted in sedation, hypotension and tachycardia (Seroquel package insert, 1997), but coma and death have occurred in adults [17].

1.2.15 Risperidone

Risperidone (Brand names: Risperdal) is an atypical antipsychotic approved by the United States Food and Drug Administration (FDA) in 1993 for the treatment of schizophrenia, schizoaffective disorder, the mixed/manic states associated with bipolar disorder (2003), and irritability in children and adolescents with autism (2006). In 2007, risperidone was approved for treatment of schizophrenia in youths ages 13–17 and for treatment of bipolar disorder in youths and children ages 10–17. Risperidone has many off-label uses such as for the treatment of various anxiety disorders, treatment-resistant depression, Tourette syndrome, disruptive behavior disorders in children, and eating

disorders [38]. Acute overdose may cause tachycardia, hypotension, akathisia, dystonia seizures and death.

1.2.16 Thioridazine

Thioridazine (Melleril, Mellaril, Novoridazine, Thioril) is a typical antipsychotic belonging to the phenothiazine drug group, synthesized in 1958 and made clinically available in 1959 for the treatment of schizophrenia, schizoaffective disorder, and short-term treatment of severe behavioral problems in children. Off-label thioridazine may be prescribed to treat acute mania, psychotic depression, and severe psychotic depression. Thioridazine, has been associated with Torsades de pointes type arrhythmias and sudden death, as a result it is reserved for use in patients who failed to respond to, or have intolerable adverse effects for more widely used antipsychotics. Patients receiving therapeutic doses have died from adverse effects (neuroleptic malignant, ventricular arrhythmias, agranulocytosis) and overdoses are common, with many of these episodes being fatal.

1.2.17 Thiothixene

Thiothixene (Navane) is a typical antipsychotic drug of the thioxanthene class that was synthesized in 1964 and made available the treatment of psychoses like schizophrenia in 1965. Thiothixene is not for use in psychotic conditions related to dementia because it may cause heart failure, sudden death, or pneumonia in older adults

with dementia-related conditions however off-label uses have included psychotic depression, agitation, and dementia. Overdose symptoms may include drowsiness, dizziness, extrapyramidal symptoms, tachycardia, hypotension, tardive dyskinesia, blood dyscrasias, and death [17].

1.2.18 Trifluoperazine

Trifluoperazine (Eskazinyl, Eskazine, Jatroneural, Modalina, Stelazine, Terfluzine, Trifluoperaz, Triftazin) is an atypical antipsychotic of the phenothiazine chemical class. Trifluoperazine was approved by the U.S. Food and Drug Administration for treatment of psychotic disorders, including schizophrenia, schizoaffective disorder, and drug-induced psychosis. It may also be used for short-term treatment of severe anxiety, agitation, violent or dangerous behavioral problems, and to treat severe nausea and vomiting. Off-label trifluoperazine may be prescribed with a mood stabilizer to treat acute mania, since the mood stabilizer has a slower onset of action. It has declined in use because of the high frequency of severe tardive dyskinesia. Overdoses with trifluoperazine can cause sedation, agitation, convulsions, fever, coma, hypotension cardiac arrest, and death.

1.2.19 Ziprasidone

Ziprasidone (marketed as Geodon, Zeldox by Pfizer) is an atypical antipsychotic that is a dopamine antagonist, chemically related to risperidone. It gained FDA approval in 2001

for the treatment of schizophrenia, mania and mixed states associated with bipolar disorder, and the intramuscular injection form was approved for acute agitation in schizophrenic patients. In 2009, the pharmaceutical company Pfizer pled guilty to illegally marketing ziprasidone (Geodon) as an effective treatment of conditions such as depression, bipolar maintenance, mood disorder, anxiety, dementia, and obsessive compulsive disorder. Pfizer sales representatives sent out unsolicited details to doctors explaining off-label uses and dosages not approved by the FDA because they were considered unsafe. (Pfizer paid $2.3 billion in the settlement) [37]. Adverse reactions to ziprasidone have included headache, sedation and postural hypotension; however, in older adults with dementia-related conditions, it may cause heart failure, sudden death, or pneumonia.

Table 3: TIAFT reference blood level list of therapeutic and toxic substances.

"TIAFT reference blood level list of therapeutic and toxic substances" September 2004

Therapeutic levels are the steady state concentrations that need to be reached for the drug to exert a significant clinical benefit without causing unacceptable side effects.

Toxic levels are serum concentrations above which unacceptable, concentration dependent, side- or toxic effects might appear.

Substance (Mol. weight) - Matrix - [Ref. Concentration **Therapeutic** mg/L (T=trough; P=peak)] - [Ref. **Concentration Toxic mg/L (T=trough; L=lethal)**];

[(10)20-40(50)]: means: normally between 20-40 mg/L, but some authors or clinicians are using ranges between 10 and 50 mg/L. * case report Bold = changed after April 2005

- Buspirone (385.5) - Serum - [0.0009-0.005(0.01)] - **[-]**
- Chlorpromazine (318.9) - Serum - [0.03-0.5; child 0.04-0.1] - **[0.5-2; L2]**
- Clozapine (326.8) - Serum - [0.1-0.6(0.8)] - **[(seizures >0.5); 0.8-1.3; L 3]**
- Fluphenazine (437.5) - Serum - [(0.0002-)0.001-0.017] - **[0.05-0.1]**
- Haloperidol (375.9) - Serum - [0.005- 0.015 (0.04)] - **[(side effect 0.01);0.05-0.1; L 0.5]**
- Loxapine (327.8) - Serum - [0.01-0.03(-0.1)] - **[1; L7.7]**
- Olanzapine (312.4) - Serum - [0.01-0.05(0.1)] - **[0.2; L 1*]**
- Perphenazine (404.0) - Serum - [0.0004-0.03] - **[0.05-0.1]**
- Pipamperone (375.5) - Serum - [0.1-0.4] - **[0.5-0.6]**
- Promethazine (284.4) - Serum - [(0.05)0.1-0.4] - **[1; L 2.4*]**
- Quetiapine (383.5) - Serum - [T (0.025)0.075-0.17; P 0.14-0.365] - **[1.8*; L 7-]**
- Risperidon (410.5) - Serum - [(0.004)0.01-0.03 (0.09)] - **[L 1.8*]**
- 9-hydroxyrisperidon (426.5) - Serum - [sum 0.015-0.06] - **[0.08]**
- Thioridazine (370.6) - Serum - [0.2-1] - **[2(5); L 3-10]**
- mesoridazine (386.6) - Serum - [0.3(0.2-1.6)] - **[-]**
- Trifluoperazine (407.5) - Serum - [(0.001-)0.005-0.05] - **[0.1-0.2]**
- Ziprasidone (412.9) - Plasma - [0.02-0.06] - **[-]**

1.3 Solid Phase Extraction (SPE)

When analyzing compounds in blood, urine or other mediums, it is important to recognize the effects of the matrix of that medium. These are called matrix effects and are the combined effects of all components in the sample other than the analyte which causes interference in the measurement of the quantity. When analyzing compounds with methods such as LC-MS(MS), especially employing electrospray ionization, ion enhancement or suppression can cause variability in results [40] [41] [42] [43]. Removing the various constituents of the matrix would reduce matrix effects, thus diminishing its impact on the measurements of the analysis and improving the results [44] [45]. This can be achieved by sample preparation with liquid–liquid extraction (LLE) or solid-phase extraction (SPE) both of which can help isolate specific compounds, remove interfering matrix components, and produce a sample that is concentrated enough for detection. When considering the analytes of this study LLE and SPE have shown similar results but SPE (Figure 6) has produced slightly better recoveries and was selected for use [46] [41] [47] [48]. SPE also offers the benefit of complete phase separations translating to cleaner extracts, a reduction in the use of organic solvents, less time and labor intensive, and easier to automate. Three basic types of SPE are normal phase, reversed phase, and ion exchange.

Normal phase uses a nonpolar liquid phase (mobile phase) and a polar modified solid phase (stationary phase) and relies on hydrophilic interactions (hydrogen bonding, polar-polar, pi-pi, dipole-dipole, and dipole-induced dipole interactions). This method

involves a polar analyte of interest and a mid- to nonpolar sample matrix. The analytes of interest are eluted by employing a polar solvent to disrupt the binding forces between the compound and the sorbent (stationary phase) [12].

Reversed phase uses a polar liquid phase (mobile phase) and a nonpolar modified solid phase (stationary phase) and relies on hydrophobic interactions (nonpolar-nonpolar and van der Waals forces). This method involves a mid- to nonpolar analyte of interest and a polar or slightly polar sample matrix. The analytes of interest are eluted by employing a nonpolar solvent to disrupt the binding forces between the compound and the sorbent [13].

Ion Exchange uses a charged group on the stationary phase's surface and relies on electrostatic attraction to retain compounds that are charged when in a solution. The choice of bonded silica cartridges is dependent on the charge of the compounds (anionic or cationic). Ion exchange SPE procedures depend heavily on the pH of the sample and conditioning solutions, so for retention of the analyte, the pH of the sample must be one at which the analyte and the functional groups on the silica surface (sorbent) are charged oppositely. The analytes of interest can be eluted by disrupting the electrostatic bond with a solution having a pH that neutralizes either the compound or sorbent's functional group. This can also be done with a solution containing an ionic species that displaces the analyte.

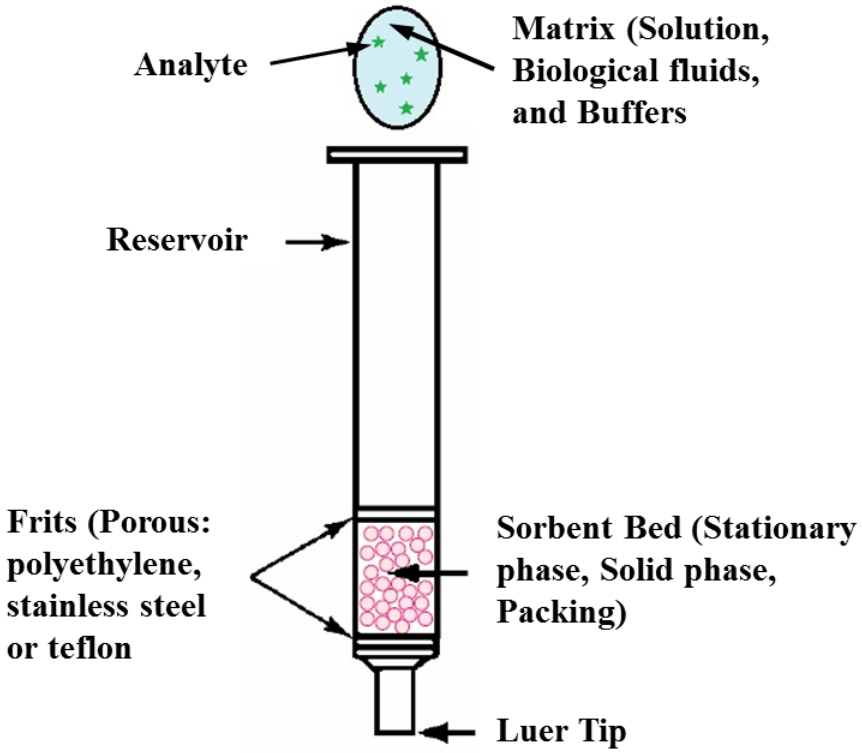


Figure 2: Components of Solid Phase Extraction (SPE)

The antipsychotics used in the study are basic compounds, therefore Ion exchange would be the preferred method of SPE and more specifically cation exchange (oppose to anion exchange) which retain positively charged cations, such as bases. When the recovery of weak cations is required, cation exchange sorbents containing silica with aliphatic sulfonic acid groups (pKa <1) are used. These groups are bonded to the surface and are charged over the whole pH range, isolating strong or weak cationic compounds

charged in solution of the correct pH range (matrix must be 2 pH units below its pKa). Weak cations can be isolated and eluted with a solution at 2 pH units above the cation's pKa (neutralizing the analyte), or by adding a different cation that displaces the analyte. Recoveries of strongly cationic species require cation exchange sorbents containing silica with aliphatic carboxylic acids (pKa 4.8). These groups are bonded to the surface and are charged in solutions of at least 2 pH units above the pKa and will isolate charged cations. Analytes can be recovered for both strong and weak cations by neutralizing carboxylic acid functional groups (2 pH units below its pKa) or employing different cations that displaces the analyte [14].

Once the appropriate column, pretreatments, solvents, and pH are determined the solid phase extraction can be performed. Ion exchange SPE procedures may require that pretreatment of the sample is done to ensure proper pH and increase SPE recoveries. The SPE tube packing may require conditioning to charge the functional groups of the stationary phase. The sample is then transferred to the SPE tubes where the solution passes through the packing while the analyte, of interest is retained on the packing. Then the solution that was used in the sample or one that will not remove the retained analytes is added to the SPE tube to wash off unwanted and unretained materials. To elute the analyte the sorbent bed is rinsed with a solution that will remove the analytes of interest leaving any additional impurities behind.

1.4 Liquid Chromatography Tandem Mass Spectrometry

Liquid Chromatography Tandem Mass Spectrometry has a wide range of functions and uses; however this discussion will be primarily limited to their capabilities, uses and applications as performed in this study. High performance liquid chromatography coupled with tandem mass spectrometry is being more commonly used for the detection of antipsychotic drugs in many biological mediums [46] [50] [53] [54] [55] [56] [57] [58] [59]. In forensic toxicology, there is a need for the detection of low-dose antipsychotic drugs in postmortem blood which requires a high degree of sensitivity and selectivity. Other methods such as GC-MS and gas chromatography coupled with nitrogen phosphorous detection (GC-NPD), require thermal stability of the drugs and demonstrate inferior sensitivity and selectivity [60]. Liquid chromatography systems coupled with other detection methods such as UV detection (HPLC-UV) have also yielded inferior sensitivity and selectivity [61] [60]. LC–MS(MS) provides the superior sensitivity and selectivity required for the detection of low-dose antipsychotics in postmortem blood and is the best choice for this type of analysis [50] [62] [63] [55].

1.4.1 High Performance/Pressure Liquid Chromatography (HPLC)

In the early 1900s, Russian botanist, Mikhail S. Tswett separated compounds (leaf pigments), extracted from plants using a solvent, and a column packed with particles. He filled an open glass column with specific types of particles and poured his solvent extract of plant leaves into the column followed by pure solvent. As the sample passed down

through the column by gravity, different colored bands could be seen separating because some components were moving faster than others. The compounds that were more strongly attracted to the particles slowed down, while other compounds more strongly attracted to the solvent moved faster. To describe his colorful experiment Tswett coined the name chromatography or liquid/column chromatography. The acronym HPLC was coined by a chemical engineer and physical chemist named Csaba Horváth in 1970, for his work in liquid chromatography where a pump (rather than gravity) provides the higher pressure required to move the mobile phase and analyte through the densely packed column. With the advances in performance such as smaller particles and higher pressures, the acronym was changed to high performance liquid chromatography.

Liquid chromatography separation occurs based on the interactions of the sample with the mobile and stationary phases and many stationary/mobile phase combinations can be used to separating a mixture. There are four basic types of chromatography in which the mobile phase is a liquid: adsorption chromatography (liquid-solid); ion-exchange chromatography, size exclusion chromatography (gel), and partition chromatography.

Adsorption, or liquid-solid, chromatography was discussed at the beginning of this section because it was what Tswett discovered. It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase of silica or alumina. Their adsorption characteristics are similar, however, alumina offers the advantages of higher sample capacity and its wider range of useful forms [27]. The choice of the mobile

phase composition is very important since it is the only variable able to be optimized and the equilibration between the mobile and stationary phase will determine the separation characteristics of different analytes. Absorption would not be useful when considering the number of analytes, the small amount, and possible pH restrictions. However, the sample collection and analysis alone make this method a nonviable option.

Ion-exchange chromatography (IC), separates analytes based on their respective charges by employing a charged stationary phase to separate charged compounds. In conventional methods, HPLC columns packed with anion-exchange or cation-exchange resins carry charged functional groups which interact with oppositely charged groups of the compound to be retained. This method would not be useful in this study because the analytes are not ionic species.

Size-exclusion chromatography SEC (gel permeation - organic solvent used, gel filtration - aqueous solution used) is primarily used with high-molecular-weight species, separating molecules according to their size (hydrodynamic volume). Packings for size-exclusion chromatography consist of silica or polymer particles containing uniform pores into which solute and solvent molecules can diffuse. Molecules that are larger than the average pore size of the packing are not retained (excluded) and elute first. Molecules with diameters smaller than the pores can enter the pores and depending on how small they are, can spend more time navigating through the maze of pores and are last to be eluted. This method is not appropriate for this study due to it requirement of a least a 10% difference in molecular weight for reasonable resolution in addition to the restriction on

the number of analytes because of the short time scale [27].

Partition chromatography is the most widely used of all of the four types of liquid chromatographic procedures and is the type used in this study. Within partition chromatography, there is liquid-liquid and bonded-phase chromatography where molecules equilibrate (partition) between a liquid stationary phase and the mobile phase. In liquid-liquid, the stationary phase is held on the surface of the packing by physical adsorption. However, this suffers from disadvantages such as loss of stationary phase by dissolution in the mobile phases, which further prohibits the use of liquid-phase packings for gradient elution due to solubility issues. Bonded-phase is primarily used where the stationary phase is chemically bonded to the support surfaces. There are two types of partition chromatography distinguished based upon the relative polarities of the mobile and stationary phases which are normal-phase and reversed-phase chromatography.

The term “normal” in Normal-phase HPLC came from the fact that in the 1970s most liquid chromatography was done in this way with non-bonded silica or alumina with its stronger affinity for polar compounds was useful in separating species based on polarity. Normal-phase HPLC (NP-HPLC), or adsorption chromatography uses a polar stationary phase and a non-polar, non-aqueous mobile phase to separate analytes that are soluble in non-polar solvents retaining more polar analytes by the polar stationary phase. The strength with which analytes are retained depends on their polarity, steric effects, the polar stationary phase, and the degree of polar solvents in the mobile phase. Therefore, in non-polar solvents, the least polar analytes are more soluble so they have a lower

retention time and are eluted first. By increasing the polarity of the mobile phase, the least polar analytes are less soluble and polar solvents tend to deactivate the polar stationary phase so they have a higher retention times, decreasing the elution time. The problems with normal-phase chromatography are retention times may be variable and gradient elution can be difficult because of water adsorption by the commonly used silica columns. The problems with alumina are it has a low theoretical plate number (N), variable retention times, and low sample recovery [27] [63].

Most high performance liquid chromatography is currently performed on columns with reversed-phase packings. Reversed phase HPLC (RP-HPLC or RPC) has a bonded non-polar stationary phase and an aqueous, relatively polar mobile phase. Alkyl chains are bonded covalently to the support surface to reverse the elution order so polar compounds are eluted first hence the term ‘reverse”. Mobile phases usually consist of a mixture of water or aqueous buffers and various concentrations of organic solvents miscible in water such as methanol, acetonitrile or tetrahydrofuran (THF). The addition of organic solvents to water or aqueous buffers can change retention times by making the mobile phase more hydrophobic, decreasing the affinity of the hydrophobic analytes to the hydrophobic stationary phase (de-sorption). Reversed-phase chromatography works primarily by hydrophobic forces which result from the polarity of water and the subsequent affinity of the hydrophobic surfaces of the analyte to the hydrophobic stationary phase. There are a few primary factors driving the separation and affecting the analyte retention characteristics. The primary factors driving the separation and affect the analyte retention characteristics are: the structural properties of the analyte such as its

hydrophobic surface area and polar groups; longer hydrophobic chains in packings which are more retentive towards hydrophobic analytes; and, the addition of mobile phase modifiers, pH and manipulating the concentrations of water and organic solvent (gradient elution). Buffers serve multiple purposes in the mobile phase by controlling pH, neutralizing any charges on exposed silica in the stationary phase and acting as ion pairing agents to neutralize charges on the analyte. The mobile phase (A) is generally prepared with strong acid. This low pH environment suppresses the ionization of the acidic groups in the analyte molecules and, therefore, changes their retention characteristics. Silanol groups on the surface of silica-based media, which will be discussed in the section on the columns, can arise from inadequate end-capping and column aging. This can cause a negative effect called mixed mode retention which can be eliminated by creating low pH environment causing suppression of the ionizable silanol groups. Acids such as acetic acid or formic acid are used to improve the chromatographic peak shape and to provide a source of protons, if mass spectrometry is used to analyze the column effluent. Ammonium formate can be used as a buffer and is commonly added in mass spectrometry to improve detection of certain analytes by the formation of ammonium adducts. Elution can be performed isocratic where the mobile phase composition remains constant throughout the separation process or by using a gradient, where the mobile phase composition is changed during the separation process. Gradient elution tends to be the method of choice because separation efficiency is greatly enhanced. Gradient elution proceeds from a condition of high polarity to low polarity so the concentration of organic solvent is lower in the initial mobile phase (A) than it is in the final mobile phase (B). Mobile phase (A) often water or an aqueous buffer and mobile

phase (B) an organic solvent, are varied continuously or in a series of steps to form the gradient slope which are combinations of a linear gradient and isocratic conditions. Gradient elution decreases the retention of the later-eluting components, improves tailing and produces narrower taller peaks for many analytes. In general, decreasing gradient slope increases resolution, however, peak volume and retention time increase with decreasing gradient slope so the gradient shape should be adjusted to optimize the separation of the desired components.

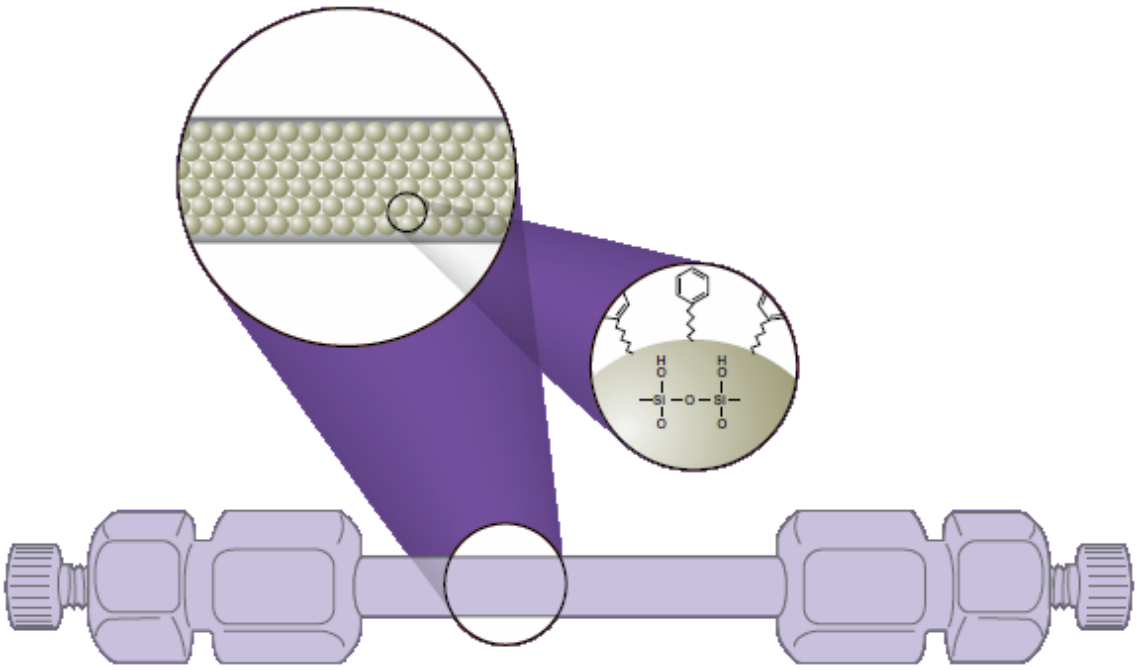
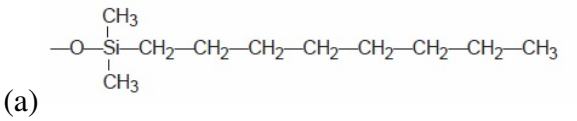
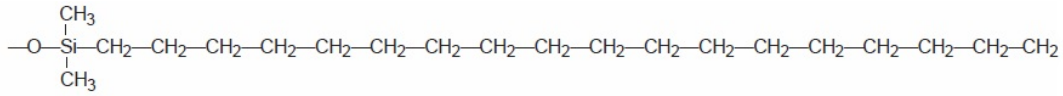


Figure 3: HPLC Column showing solid phase and surface chemistry (Phenomenex)

When determining a column for reversed-phase chromatography the important characteristics to evaluate are chemical composition of the packing, pore and particle size of the beads, the type and density of ligand bond to the surface of the bead, and the capping chemistry. The base packing media used is normally silica produced as porous beads which are chemically stable at low pH (dissolve at high pH) and in the organic solvents. The chemistry of the silica gel allows simple derivatization with ligands of various carbon chain lengths and it is these alkyl groups that are predominantly responsible for its analyte retention properties (Figure 3). Many columns are composed of siloxanes bonded to an octadecyl carbon chain (C18) although there are others carbon chains such as C8 and phenyl bonded silica (Figure 4).



(a)



(b)

Figure 4: Carbon chains that are bonded to the siloxanes of the solid phase, (a) octal carbon chain (C8) (b) octadecyl carbon chain (C18)

However, due to steric hindrance, not all silanol is treated and a secondary bonding step to cover unreacted silanols on the silica surface is needed. These untreated or unreacted silanols cause undesirable polarity to the surface called mixed mode retention. Mixed-mode retention causes skewed and broader peaks, and retention times increase by ion exchange interaction. End-capping is a process which unreacted silanols are treated with short-chain organosilanes (Figure 5) (Figure 6).

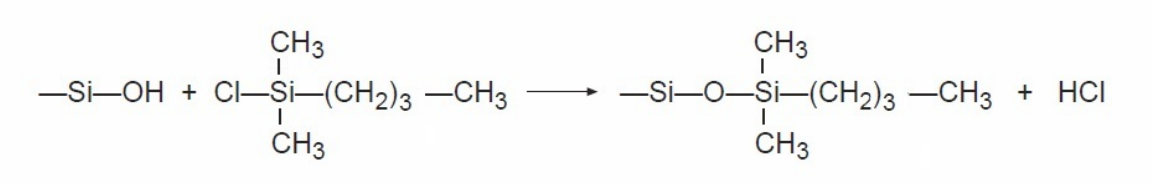


Figure 5: End-capping: The bonding process of the carbon chain to the unreacted silanols of the solid phase

The choice of the alkylsilane reagent to treat these sites will change the selectivity of the column allowing for variation among packaging with the same alkyl groups. So although it is not possible to predict which ligand will be best for a particular application there are varying degrees of selectivity that can be achieved through different C18 columns alone. When examining column size it can be said that for the same packing

material, shorter columns would provide faster run times, while longer columns provide better resolution.

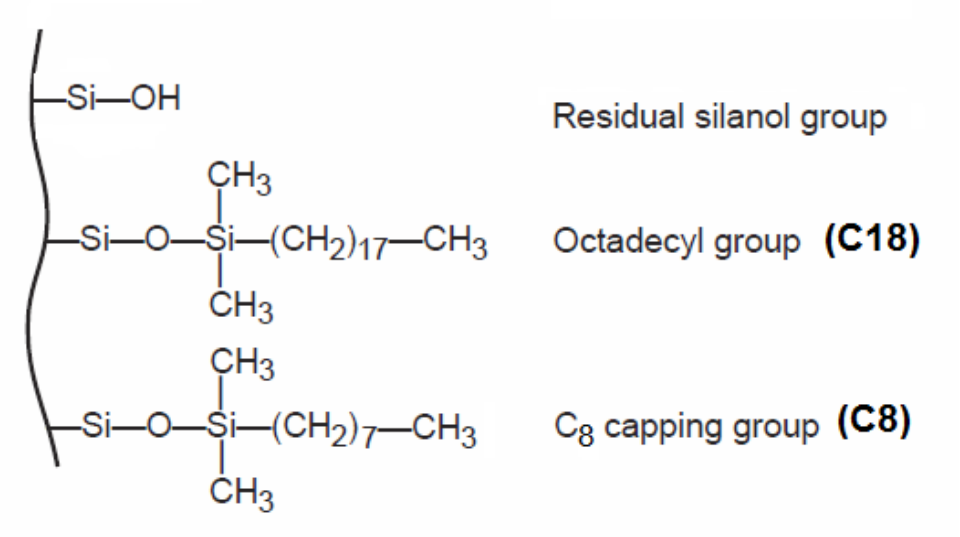


Figure 6: Solid phase with an unreacted silanol, C18, and C8 group attached

There are many factors to consider such as the characteristics of the packing material, and the relationship between column length, diameter and volume. The resolution of high molecular weight biomolecules in reversed phase separations is less sensitive to column length than is the resolution of small organic molecules. However, the use of gradient elution reduces the significance of column length with regard to

resolution although longer columns with small diameters often provide increased resolution, they will have lower sample loading capacity and higher back-pressures requiring modifications of other HPLC parameters. HPLC prefilter cartridge systems can be employed to protect the HPLC columns from contaminants, and chemical damage without altering your chromatographic results (Figure 7).

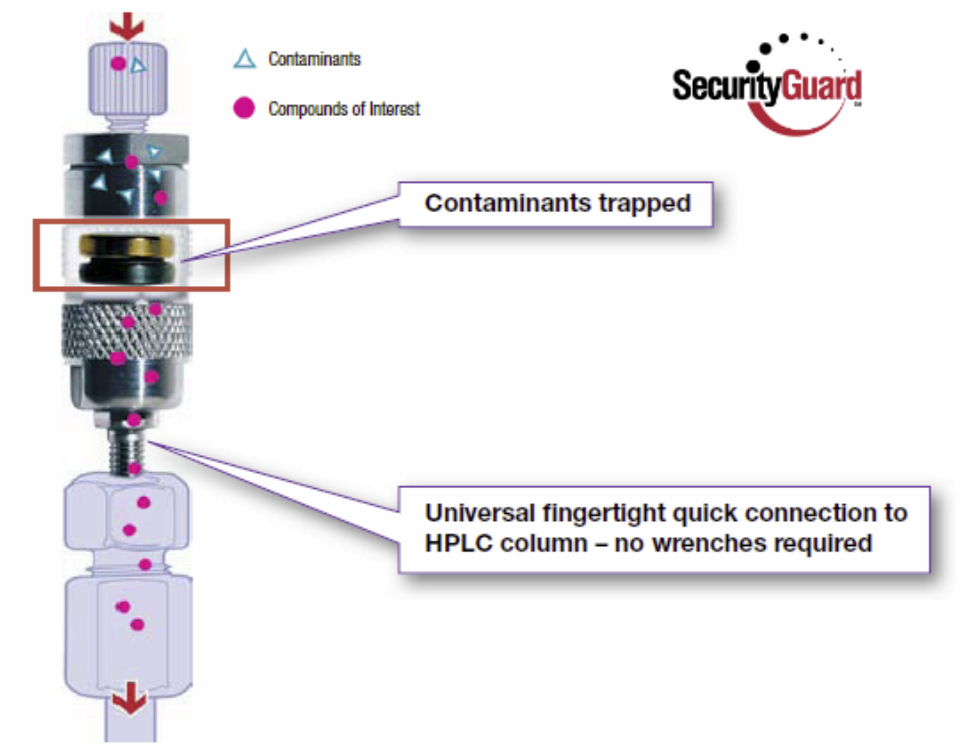


Figure 7: HPLC prefilter that is attached to the front end of the column (Phenomenex)

Other parameters to consider in reversed phase chromatography are flow rate, temperature, and mobile phase. Flow rate is not as critical during analytical experiments as large scale preparative reversed phase chromatography. However other factors such as column size, stationary phase particles size and temperature affect the flow rate. Increasing flow rate can impact analysis time, quality of the chromatography, and exceed pressure limitations of the system. The viscosity of the mobile phase decreases with increasing column temperature. Decreasing solvent viscosity has the effect of reducing pressure when high pressures become a concern. Further, mass transfer between the mobile and stationary phase becomes more efficient leading to higher resolution.

HPLC is equipped with one or more glass or stainless steel reservoirs that hold the solvents. The reservoirs are equipped with degassers to remove dissolved gases that would form bubbles in the column and the detector systems and cause band spreading and interfere with the detector. Proportioning valves select solvents and change the amounts of the mobile phase over time in gradient elutions. The solvent delivery system is a high-pressure pump used to produce a specified flow rate of mobile phase and it is necessary to pump the eluent at a constant flow rate and pressure. An autosampler is equipped with six-port valves; so that a sample can be injected into the flowing mobile phase stream at continuous pressure into the HPLC column flowed by the detector (Figure 8).

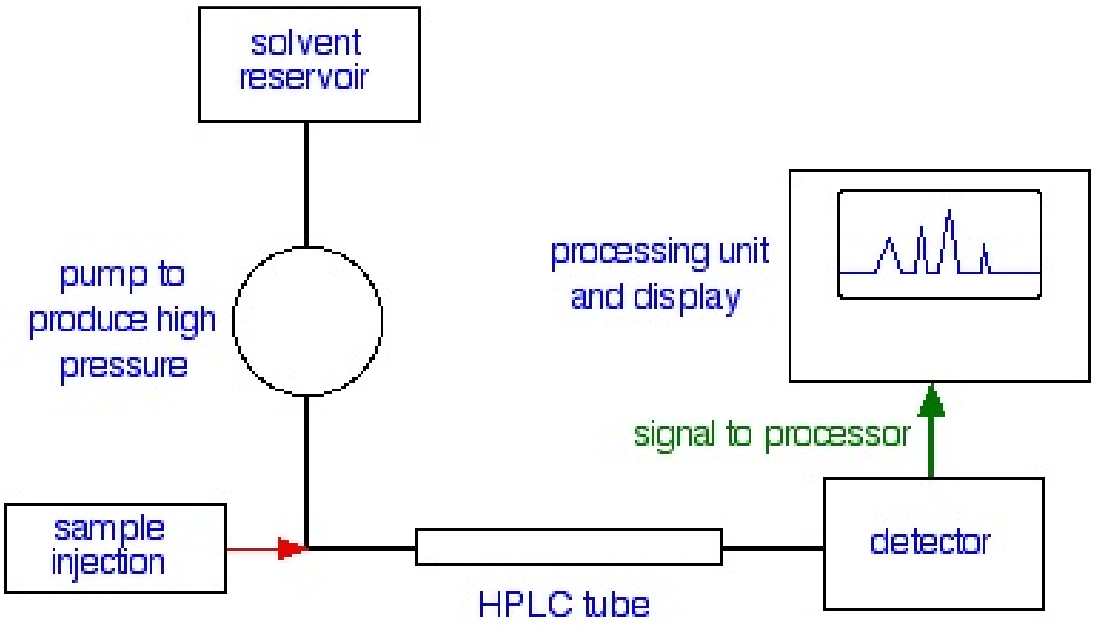


Figure 8: HPLC block diagram

1.4.2 Electrospray Ionization Tandem Mass Spectrometry

Ionization is required in mass spectrometry because only ions can be accurately measured and electrospray ionization is useful because molecules being ionized are not broken apart, providing a known ion mass to use to determine the chemical composition (soft ionization). It forms ions of multiple charges making their mass-to-charge ratio small enough to be detectable, especially in the analysis of large molecules such as proteins. Atmospheric pressure ionization (API) or in particular electrospray ionization

(ESI) is a technique used in mass spectrometry to produce ions and is ideal for LCMS systems because analysis can be performed with direct sample introduction from the HPLC column directly to electrospray ionization source. A liquid containing the analyte(s) of interest is pumped through a stainless steel capillary needle and a very high voltage is applied to the sharply pointed tip. The needle electrically charges the liquid to a very high voltage creating highly charged molecules all with the same charge. This causes the liquid to blow apart into fine aerosol of tiny, highly charged droplets sometimes with additional nebulization by an inert gas such as nitrogen. Under a field and pressure gradient, the droplets move through the interface where it is heated and nitrogen gas pumped to aid further solvent evaporation. As the solvent evaporates from the charged droplets, they decreases in size, and become unstable. When the electrical charge reaches a critical state, its Rayleigh limit, the droplet violently blows apart (Coulomb force). This continues until the solvent is completely evaporated and the droplets have split up to the point that each is a single, charged molecule (Figure 9).

Modifying the mobile phase can also help to change the types of ions produced. Both positive (addition of a proton $[M + H]^+$) and negative-ion (removal of a proton, $[M - H]^-$) spectra can be obtained by adding an acid or base to the mobile phase. Adding formic acid or acetic acid will promote protonation in the positive-ion mode and NH_4OH for deprotonation in the negative-ion mode. Also adding compounds such as acetic acid have the benefit of increasing the conductivity and decreasing the initial droplet size.

To achieve optimum ion production the ion source parameters; ionspray voltage (IS), ion source gas 1 (GS1), ion source gas 2 (GS2), temperature (TEM), and curtain gas (CUR) should be optimized (Figure 9). IonSpray Voltage (IS) is the voltage applied between the needle and orifice plate that "ionizes" and nebulizes the liquid containing the analyte(s) of interest. The polarity of the applied voltage determines what type of ions will be produced (positive or negative ions). Ion Source Gas 1 (GS1) controls the flow of the nebulizer gas that facilitates droplet formation and is optimized based upon the flow rate of the liquid. Temperature (TEM) is the temperature of the heater that heats the gas (GS2) to promote desolvation of the liquid and is optimized based on flow rate and mobile phase composition. Ion source gas 2 (GS2), increases ion efficiency with heated gas that intersects nebulized liquid stream at about 90° right in front of the curtain plate to aid in solvent evaporation. This parameter is also optimized based on flow rate. Curtain gas (CUR) is composed of high purity nitrogen that flows between the orifice and the curtain plate to prevent large droplets and neutral compounds from entering Q0. This is normally set to the highest setting before loss of the signal is seen (loss of analyte).

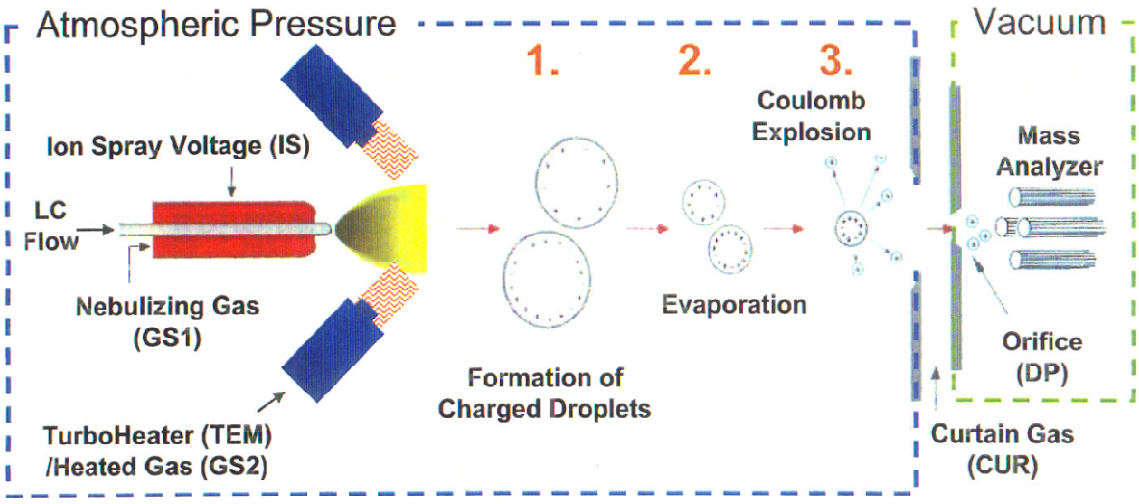


Figure 9: Electrospray Ionization (ESI) Process (AB Sciex)

A Quadrupole is a mass analyzer that uses an electric field to separate ions. A quadrupole consists of four parallel cylindrical metal rods with adjacent rod pairs

connected together electrically with opposite voltage polarity. One pair is attached to the positive side of a variable DC source and the other pair to the negative terminal both superimposed on a variable radio frequency (RF) voltage 180-deg out of phase between adjacent rod pairs (Figure 10).

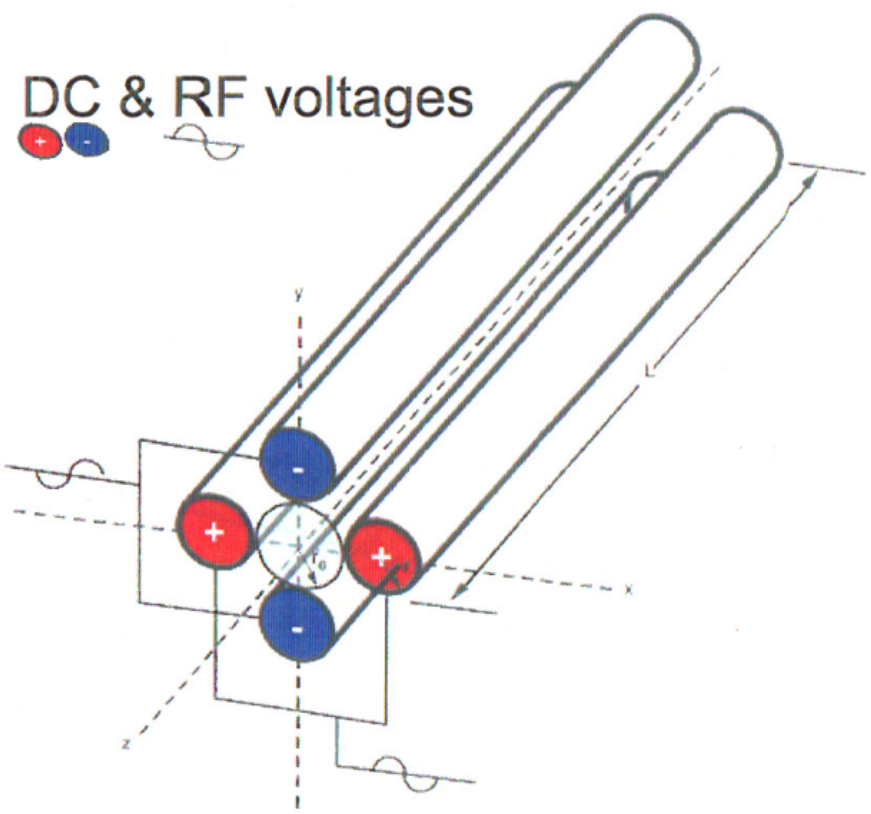


Figure 10: Quadrupoles with DC and RF voltages of the mass spectrometer (AB Sciex)

The voltage and radio frequency on the quadrupole, causes the ions to oscillate, orbit, and/or accelerated down the quadrupoles between the rods according to their mass-to-charge ratio m/z. Ions not in the range of the selected mass-to-change ratio will strike the rods and are converted to neutral molecules, while ions with a particular mass-to-charge ratio selected by the operator continue towards the detector. Quadrupole instruments can resolve ions that differ in mass by one unit and have greatly improved resolution and peak shape in recent decades.

A linear series of three consecutive quadrupoles is known as a triple quadrupole mass spectrometer or tandem mass spectrometer. The first quadrupole (Q1) serves as mass filter which is set across a preset m/z range and select an ion of a known mass to send to the second quadrupole. The second quadrupole (q2) is RF-only (non-mass filtering) and acts as a collision cell where the selected ions are broken into fragments introducing an inert collision gas into its flight path such as nitrogen. The fragments are then passed through the third quadrupoles (Q3) which like Q1 serves as mass filter, where they may be filtered or fully scanned for a tandem MS fragmentation pattern.

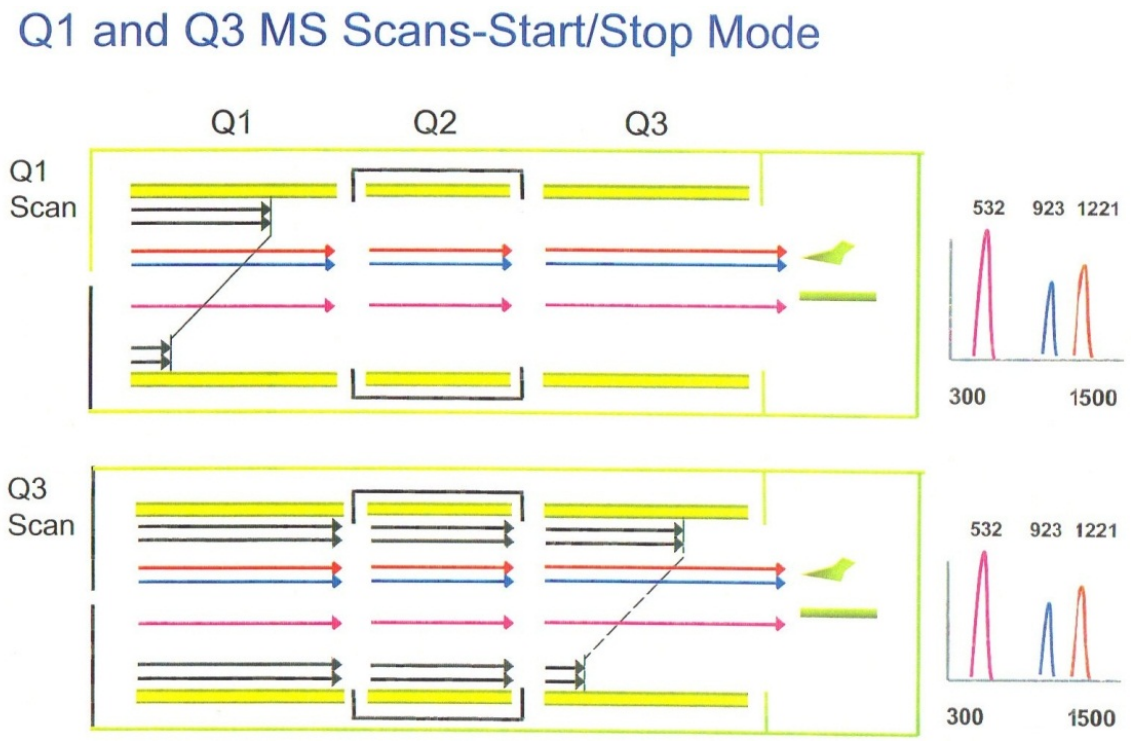


Figure 11: Full scan mode to determine precursor ions within a selected range

In a triple quadrupole mass spectrometer, there are several types of scan modes that are possible. A single scan where ion sources can be passed through the quadrupoles without collision to the detector can yield molecular weight information (Figure 11). There are four main scan modes that can be performed. In a product ion scan, product ions are formed by collisions of the precursor ions and give qualitative structural information. In a product ion scan, the first quadrupole (Q1) selects an ion of a known m/z and it is passed into Q2 were fragments are formed by collisions. The third quadrupole (Q3) scans the entire m/z giving a MS/MS spectrum for information on the

sizes of the fragments. From this information, the structure of the original ion can be determined and it also can serve as a qualifier, therefore, so even if two precursor ions have the same m/z they can be differentiated by their product ions.

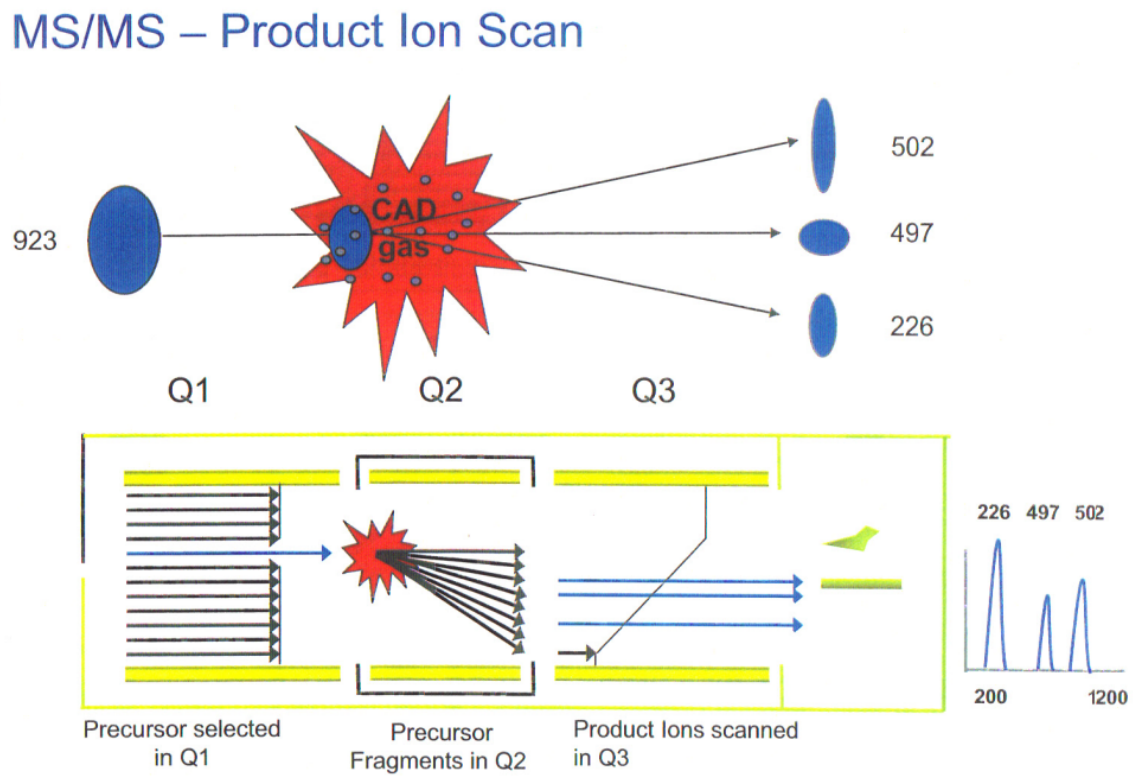


Figure 12: Steps of the Product Ion Scan in the triple quadrupole mass spectrometer (AB Sciex)

A precursor ion scan provides structural information on the source of a specific product ion by choosing a product ion and determining the precursor ions. In this scan type, Q1 is scanned across an m/z range of precursor ions and the ions are fragmented in the Q2 to produce the product ions. Q3 is set to transmit only ions of a known m/z. Therefore, all precursor ions that are passed through Q1 and fragment to produce ions with the selected m/z are detected (Figure 13).

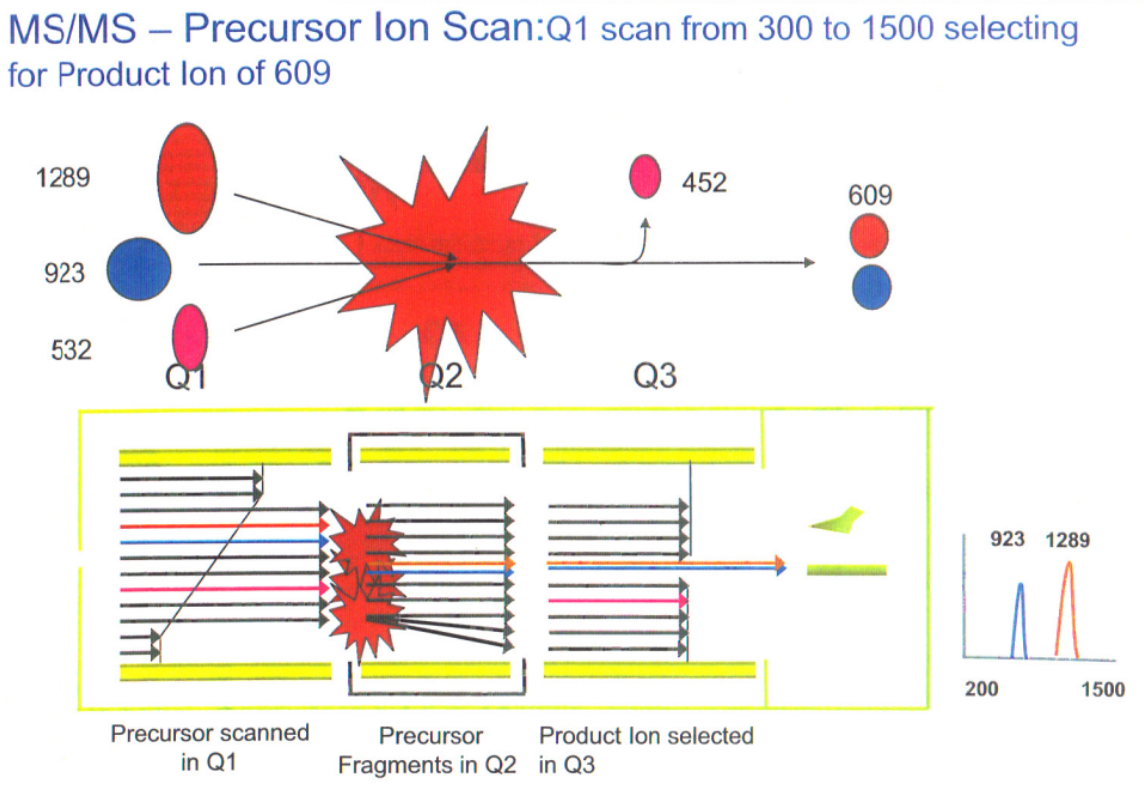


Figure 13: Steps of the Precursor Ion Scan in the triple quadrupole mass spectrometer (AB Sciex)

Neutral loss scans provide compound class specificity by screening for ions that undergo a common loss. In a neutral loss scan Q1 is scanned across a mass range of precursor ions, and the ions are fragmented in Q2 to produce the product ions as with the precursor ion scan. However Q3 selects ions of a specific m/z off set by the loss of a selected neutral fragment mass, allowing selective identification in a class of closely related compounds (Figure 14).

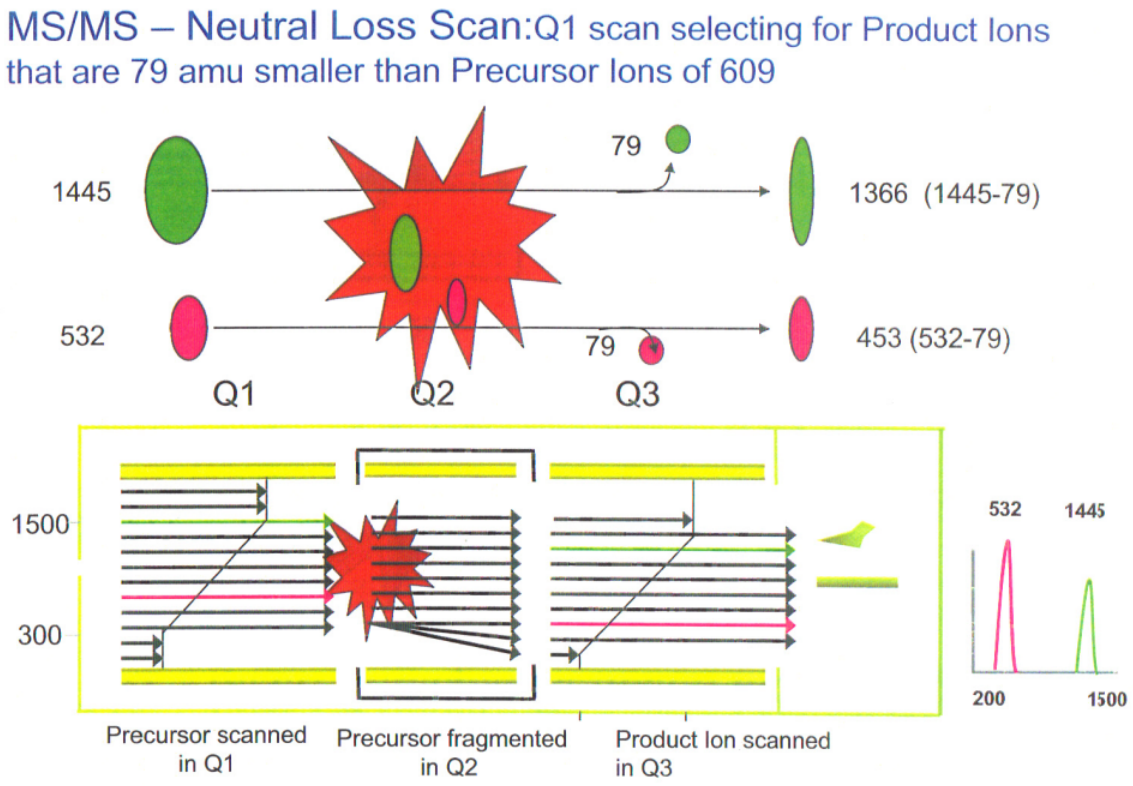


Figure 14: Steps of the Neutral Loss Scan in the triple quadrupole mass spectrometer (AB Sciex)

Multiple Reaction Monitoring (MRM) or Single Reaction Monitoring (SRM) is used for quantitation where only the ions of interest are monitored resulting in a very sensitive scan. . Both Q1 and Q3 are set to a selected m/z so the scans are focused on the precursor and product ions, increasing sensitivity. In this mode the Q1 selects an ion (SRM) or ions (MRM) of a known m/z and they are passed into Q2 where fragments are formed by collisions. Q3 is set to transmit only selected ions of a known m/z. Ions selected by the first mass analyzer are only detected if they produce the selected

fragments in Q3 (Figure 15).

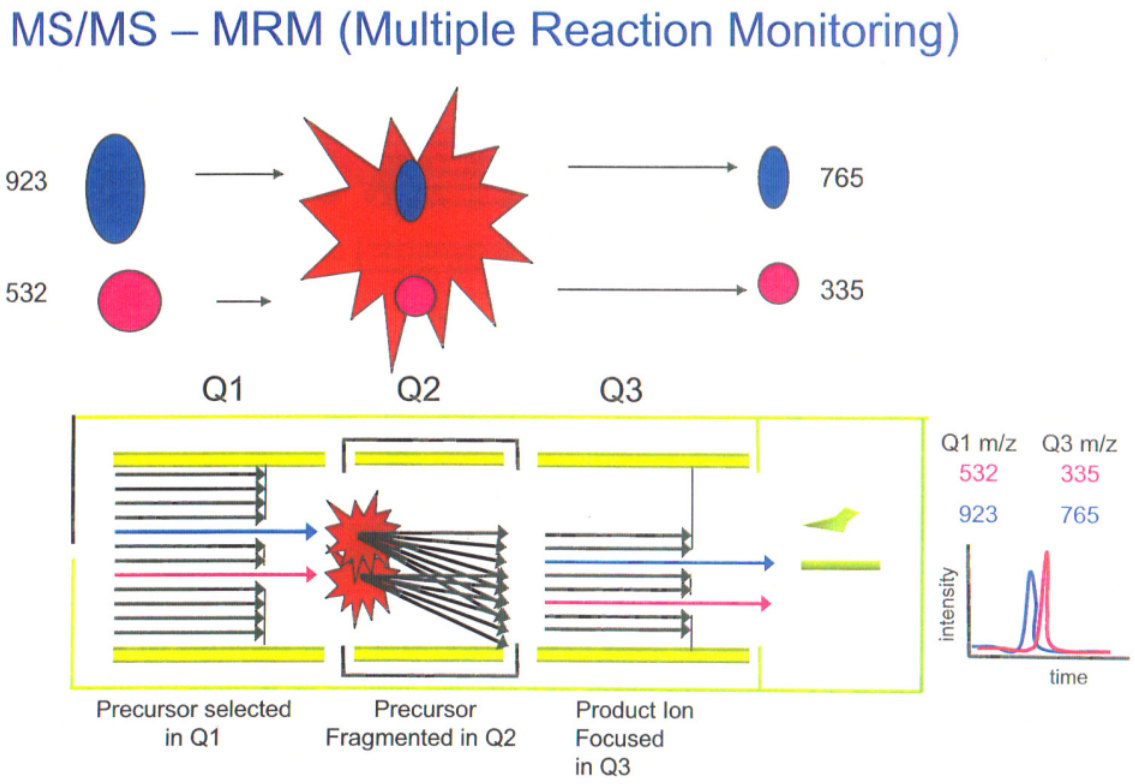


Figure 15: Steps of the Multiple Reaction Monitoring (MRM) Scan in the triple quadrupole mass spectrometer (AB Sciex)

Once the analytes are introduced in the mass analyzer, compound optimization parameters have to be adjusted to achieve the optimum selectivity and sensitivity. Declustering Potential [DP] is the potential applied to the orifice plate (OR) that breaks clusters such as $[M+H3O]^+$, $[M+Na]^+$ in the orifice region of the interface. DP is typically set to a value between 0 to 100 V, however if it is set to high it can fragment the compound of interest. Entrance Potential [EP] guides and focuses the ions through the high-pressure Q0 region and is typically set at 10 V in positive ion mode. Collision Cell Entrance Potential [CEP] is generally the most mass dependent parameter and controls the potential difference between Q0 and IQ2 focusing ions into Q2 (collision cell). Collision Energy [CE] is a voltage difference between the Q0 and Q2 and controls the energy of the collision of precursor ion with low pressure N2 in the collision cell. The larger the energy, the greater the fragmentation, dictating the nature and abundance of the product ions produced. The Collision Energy can be optimized for each analyte and produce the maximum amount of the selected product ion. Collision Cell Exit Potential [CXP] is the potential difference between Q2 and IQ3 focusing ions into Q3 (Figure 16).

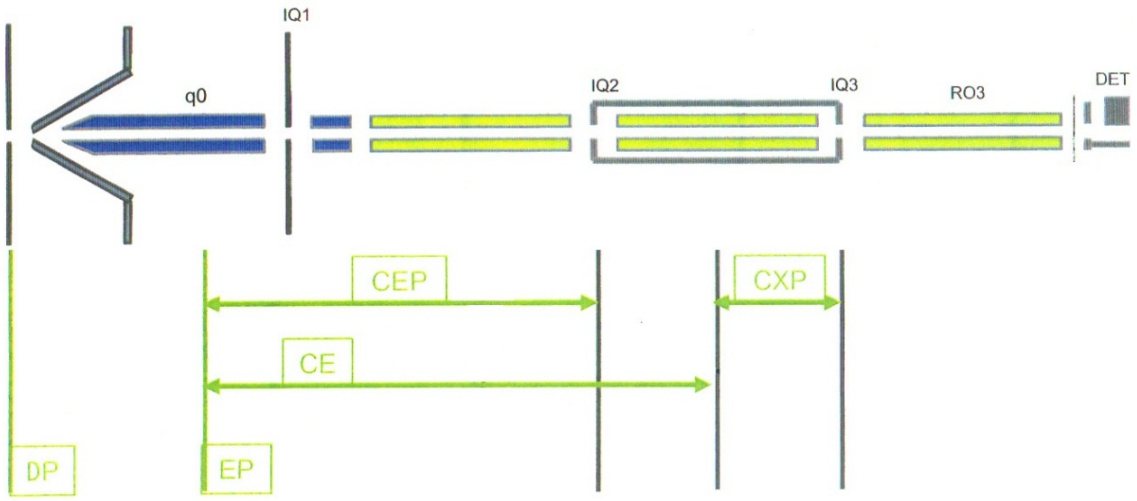


Figure 16: Zones of the triple quadrupole mass spectrometer showing the area the various energies and potentials are active (AB Sciex)

1.5 Method Development and Validation

Method Development and Method Validation in analytical toxicology are extremely important in forensic and clinical toxicology as well as quality assurance, accreditation, and publication. Well-characterized and fully validated analytical data are necessary for generating reproducible and reliable results that can be correctly interpreted and which objectively demonstrate their applicability for the intended use. Analytical data of toxicological findings in forensics must withstand the scrutiny of court to avoid being contested and could lead to unjustified legal consequences. Proper method

development and validation are imperative for an analytical method to be used in forensic or clinical toxicology. Method development is much more important to ensure the quality of an analytical method; however validation can objectively demonstrate this quality by fulfilling acceptance criteria. Method development is as the name implies the development of a method. Therefore, in chromatography it is the setting up of an analytical procedure appropriate for the analysis of a particular sample. This includes much of what is discussed in this study such as the choice of the purification and separation techniques, (i.e.; LLE or SPE and GC or LC), stationary/mobile phase, type of detector, internal standards, gradient elution, temperature programming, detector sensitivity etc... Some of these choices are determine based on current scientific analyses, what resources the lab already has and what can be obtained.

Bioanalytical method validations demonstrate that, given a biological matrix such as blood, plasma, serum, or urine, a particular method for quantitation is reliable and reproducible for the intended analytical applications. Typical parameters for this type of validation include: accuracy, precision, selectivity, sensitivity, reproducibility, and stability which can be determined through different tests.

Selectivity is the ability of a method to determine and/or quantitate target analytes in the presence of other compounds and components endogenous to the matrix without interference. Selectivity is usually concentration dependent and is determined by comparing blank samples without analytes or intended Internal Standard (IS)) of biologic matrices to determine if any potentially interfering substances are present. When

employing deuterated internal standards it is also necessary to compare samples of the biologic matrix with IS only, and with analytes only, to check for the absence of analyte fragmentation ions in the IS peak or vice versa.

Linearity or calibration model is a measure of the ability of the method to provide data proportional to the concentration of analytes within samples within a given range. This is done by the analysis of multiple replicates at different levels to identify outliers or variations across the calibration range. Accuracy (bias) and precision analysis of the data is also examined among the replicates relative to calibration range and the data is evaluated to determine if it fits a linear or non-linear model.

In this study the limit of detection (LOD), lower limit of quantitation (LLOQ) and the upper limit of linearity (ULOL) are defined as the lowest and highest calibrator used in the assay. The successful validation of these parameters is dependent on the success of the calibration model.

Accuracy (bias) is how close the measured analyte concentration of an assay matches the "true" concentration present in the sample and precision is the variability of the measurement about a mean. Accuracy (bias) and precision of the method is determined by analysis of replicates of the calibration range within and between days.

In recovery and matrix effect, analyte recovery is measured by comparing the data obtained from a known amount of analyte added to the matrix prior to extraction

compared with the data obtained from the same amount of analyte added to the matrix after the extraction. This technique attempts to produce the same matrix effects in both samples, eliminating it as a variable leaving only the change in recoveries. Matrix effect is measured by comparing the data obtained from a known amount of non-extracted analyte to the same amount of analyte added to the matrix after the extraction. This technique attempts to produce the same recovery in both samples, eliminating it as a variable leaving only the change caused by matrix effects.

CHAPTER TWO: Experimental Section

2.1 Materials and Methods

2.1.1 Chemicals and Reagents

9-Hyroxyrisperidone, Aripiprazole, Aripiprazole-D8, Chlorpromazine, Chlorpromazine-D3, Clozapine, Clozapine-D4, Fluphenazine, Haloperidol, Haloperidol-D4, Olanzapine, Promethazine, Quetiapine, Quetiapine-D8, Risperidone, Thioridazine, and Ziprasidone were purchased from Cerilliant (Round Rock, TX. USA) 9-Hyroxyrisperidone-D4, Buspirone-D8, Fluphenazine-D8, Loxapine-D8, Olanzapine-D3, Pipamperone, Pipamperone-D10, Perphenazine-D8, Promethazine-D6, Risperidone-D4, Thioridazine-D3, Trifluoperazine-D3, and Ziprasidone-D8 were purchased from Toronto Research Chemicals Inc. (North York, ON. Canada). Buspirone and Mesoridazine were purchased from W. R. Grace and Company, formerly Alltech (Cambridge, MA. USA). Loxapine was purchased from Lederle Laboratories (Pearl River, NY. USA). Perphenazine was purchased from Merck & Co., formerly Schering-Plough Corporation (Whitehouse Station, NJ. USA). Thiothixene was purchased from Pfizer Inc. (New York, NY. USA). The trifluoperazine manufacturer was unknown. Compounds and internal standards were matched as shown in Table 4. Ethyl acetate, methanol, acetic acid, chloroform, acetone, ammonium hydroxide and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ. USA). Formic acid and ammonium formate were purchased

from Sigma-Aldrich (St. Louis, MO. USA). All chemicals were of HPLC-grade or better and water was purified using the Barnstead* EASYpure* RoDi Water System (Thermo Scientific, Rydalmere, Asheville, NC. USA). Phosphate buffer (pH 6) was prepared by dissolving 27.24 g KH_2PO_4 in 1.8 L DI water. The pH was adjusted to 6 with KOH and it was filled to 2 L with DI water.

Table 4: Analytes with corresponding internal standards

Analyte	IS
Promethazine	Promethazine-D6
Olanzapine	Olanzapine-D3
Chlorpromazine	Chlorpromazine-D3
Clozapine	Clozapine-D4
Loxapine	Loxapine-D8
Thioridazine	Thioridazine-D3
Haloperidol	Haloperidol-D4
Quetiapine	Quetiapine-D8
Buspirone	Buspirone-D8
Mesoridazine	Trifluoperazine-D3
Perphenazine	Perphenazine-D8
Trifluoperazine	Trifluoperazine-D3
Risperidone	Risperidone-D4
Ziprasidone	Ziprasidone-D8
9-hydroxyrisperidone	9-hydroxyrisperidone-D4
Fluphenazine	Fluphenazine-D8
Thiothixene	Fluphenazine-D8
Aripiprazole	Aripiprazole-D8
Pipamperone	Pipamperone-D10

2.1.2 Specimens

Plastic vials for storing 6 mL of whole blood were prepared with 50 mg NaF and 10 mg $K_2C_2O_4$ in 1 L of DI water and dried. Drug-free sheep blood (with Heparin) and serum samples were obtained from Hemostat (Dixon, CA. USA) transferred to prepared plastic vials (for blood), vortexed, and filtered. Post-mortem blood samples were also placed in prepared plastic vials given to the medical examiner to obtain post-mortem blood for routine toxicological analysis in the laboratory. The post-mortem blood samples were regarded as drug free if none of the existing tests showed the presence of the studied drugs in any specimen. Classification of the post-mortem blood samples as decomposed was based upon pathology description of the body from which the specimen was taken. All blood samples were stored at −10°C prior to analysis.

2.1.3 Instrumentation

The LC-MS/MS system consisted of an Applied Biosystems 3200 Q-TRAP® linear ion-trap quadrupole mass spectrometer (AB SCIEX, Framingham, MA. USA) equipped with a Turbo V ion source, operated in the electron spray ionization (ESI) mode, and a Prominence UFLC Shimadzu HPLC system (Shimadzu Marlborough, MA. USA) equipped with a Phenomenex Synergi Hydro-RP® Polar endcapped C18 column (4 μm, 150 × 4.6 mm) and SecurityGuard® prefilter (Phenomenex, Torrance, CA. USA).

2.2 MS Method Development (Compound Optimization)

The goal of compound optimization is to determine the optimal parameters for detecting a specific compound. Stock solutions of each compound were prepared based on the original concentration purchased and in the recommended solvent for that compound, resulting in stock solution concentrations of 1000, 100, 40, and 10 µg/mL. Compounds were mixed and diluted to a reasonable concentration in a 10 mL volumetric flask. 10 µL of 1000 µg/mL stocks, 100 µL of 100 µg/mL stocks, 250 µL of 40 µg/mL stocks, and 1 mL of 10 µg/mL stocks, were all mixed in a 10 mL volumetric flask and methanol was added to the 10mL mark of the volumetric flask to make a 1 µg/mL concentration of all the compounds. 5 µL of formic acid was added to the sample to promote ion production. Compound optimization was done using the syringe pump method to obtain compound optimization information for the specific compounds. The sample mixture was loaded in a syringe and air bubbles in the syringe were removed. It was placed in the Harvard pump system of the MS and tubing was attached between the syringe and the ion source of the MS. Analyst® Software was used to ensure the S. Diameter was 4.6 and the flow rate set to 90 µL/min for the first 30 seconds, then returned to 10 µL/min for the duration of the procedure.

Analyst® software is then used to perform a Q1 MS (Q1) scan to obtain the m/z (Q1) value of the precursor ion of the analytes and/or internal standards in mixture. The scan was adjusted to start and stop 50 units above and below the molecular weight of the biggest and smallest compound. The scan time was set for 0.5 seconds each for 5

minutes. Declustering potential (DP) was set to 40 V and entrance potential (EP) to 10 V. In the spectra, the peaks represent ions with the taller peaks being the isotopic masses of the compounds. Figure 17 is a snap shot of the information that this type of scan displays. It is necessary to know the molecular weights of the compounds and then add one to this value, to account for the addition of a proton (positive ion mode) to identify the molecular ions (Figure 17).

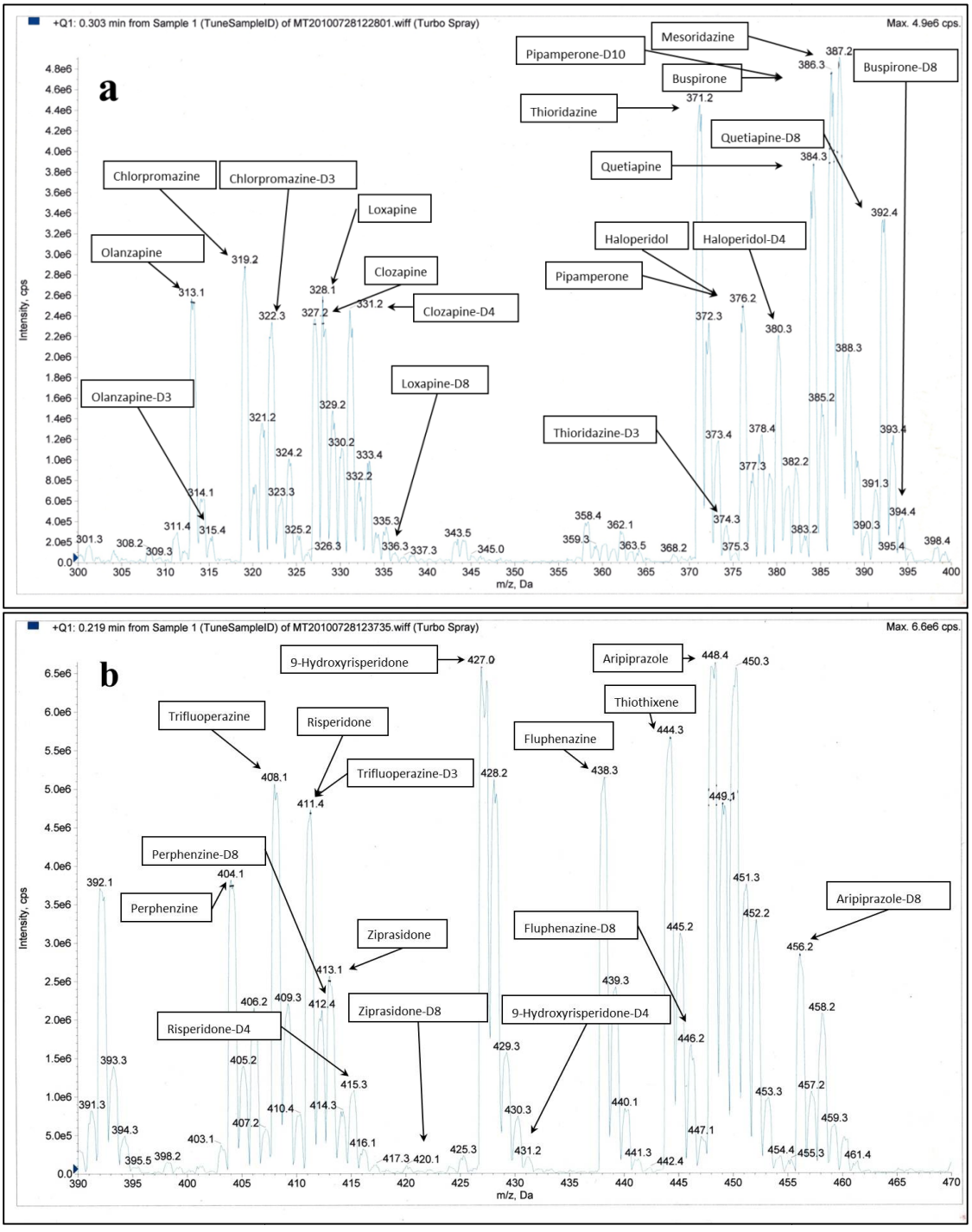


Figure 17: Snap shot of the Q1 MS scan (precursor ion chromatogram) identifying the molecular weight +1 of the compounds in the sample (a) 300-400 m/z Da, (b) 390-470 m/z Da, Promethazine and Promethazine-D6 are not shown here with m/z of 285.3 and 291.3 respectively

Once all analytes and internal standards have been identified, the m/z information can be recorded for the next step of the process. The Product Ion Scan (Q3 value) is used to determine ion products of analytes and internal standards in mixture after they are broken up with certain collision energies (CE). The m/z of one of the compounds was selected and in product ion mode, scans were done between 50 Da and slightly above the m/z of the compound. The time, duration, DP, and EP were the same as for the Q1 scan but CXP was set to 5 V and the CE was adjusted as needed, with an initial setting of 20 V. As the CE is increased, the precursor ions (Q1) decrease and the product ions increased (Q3). The value of CE chosen is the one that provides the best spectra of product ions without breaking them down too much (a good spectrum has some of the precursor ion). Figure 18 are the product ion chromatograms of 9-hydroxyrisperadone and its deuterated internal standard. The precursor ion can still be observed with two large product ion peaks however choosing the product ions was not always simple. The primary purpose of the product ion is as a qualifier, to avoid improperly identifying ions with the same or similar mass charge ratios as well as cross talk between them. Some of the compounds of this study had the m/z that only differed by 1 Da and this coupled with a poor choice of product ion in deuterated internal standards could lead to a false interpretation of the data. Figure 19 (Pipamperone) is an example of many of the same product ions between the compound and its deuterated internal standard. Pipamperone also has the same m/z of Haloperidol (376.3 Da) making the choice of ions very important.

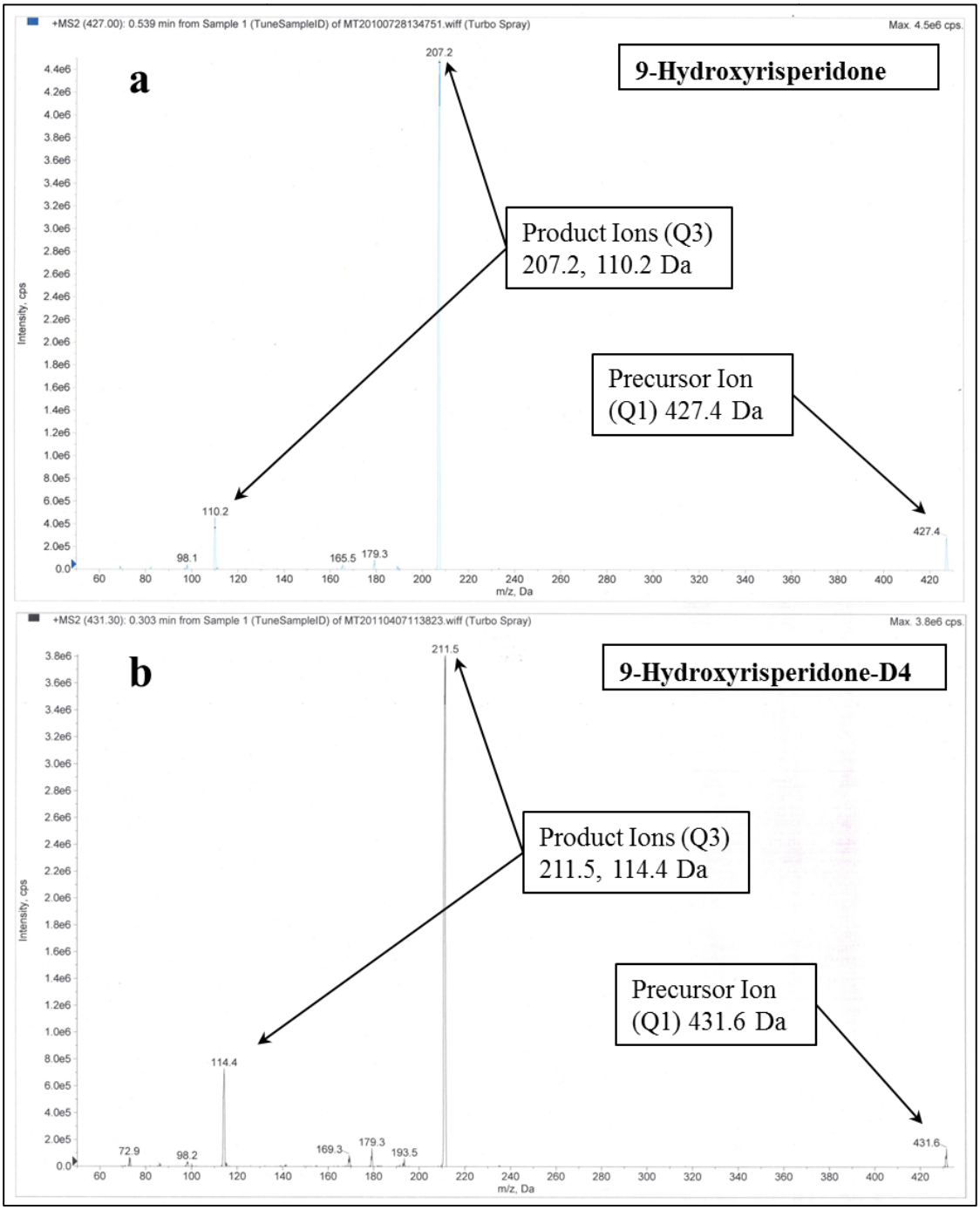


Figure 18: Product ion chromatogram of 9-hydroxyrisperidone (a) and 9-hydroxyrisperidone-D4 (b) with parent and product ions

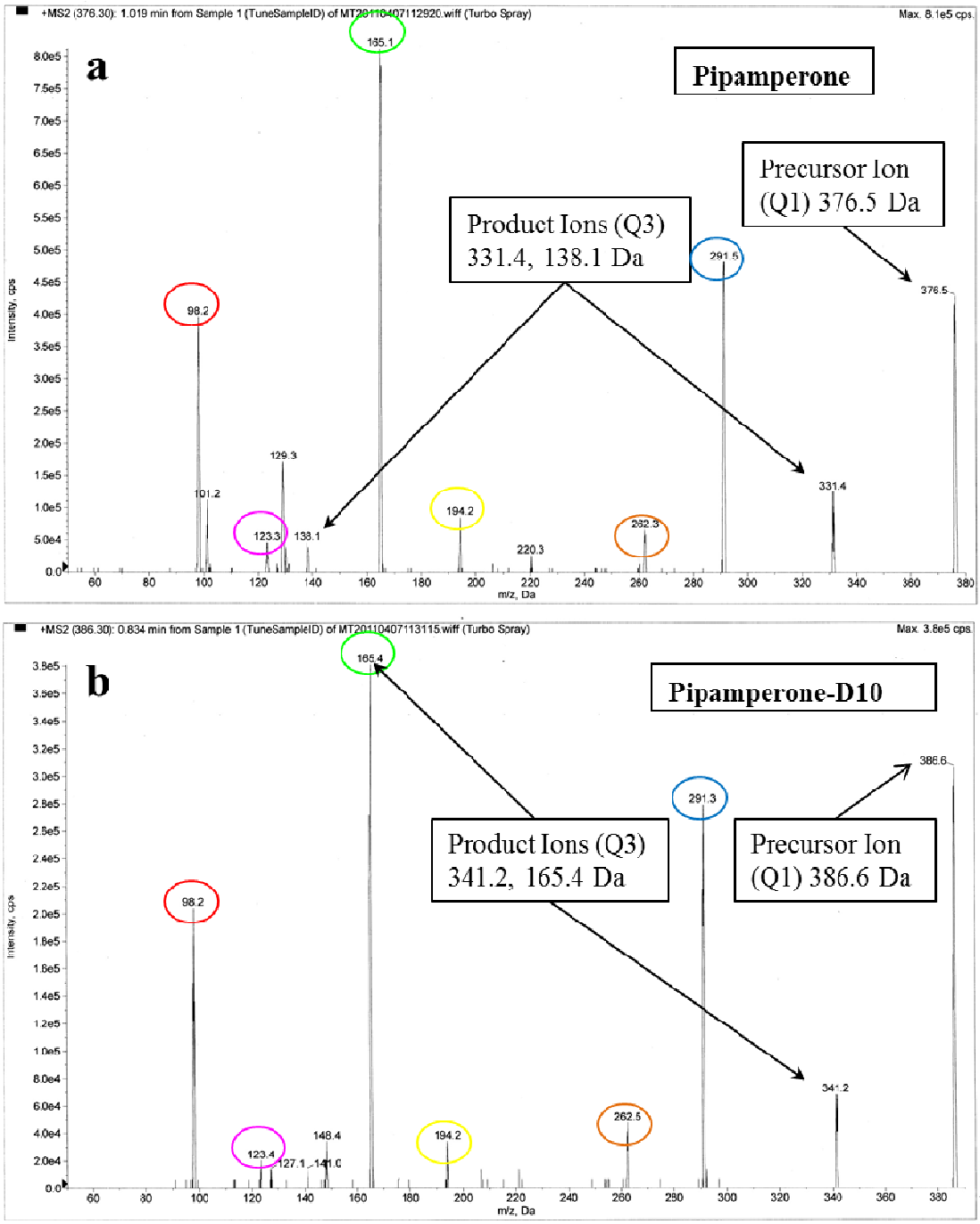


Figure 19: Product ion chromatogram of Pipamperone (a) and Pipamperone-D10 (b) with colored circles showing common ions among the compound and its deuterated counterpart, adding to the difficulty of choosing product ions for each compound

MRM method is done using the m/z of the mixture components and their chosen product ions to determine the optimum Collision Energy (CE), Declustering Potential (DP), Entrance Potential (EP), Collision Cell Entrance Potential (CEP), and Collision Cell Exit Potential (CXP) for each precursor/product ion pair. The values of precursor and product ion pairs were entered with a scan time of 150 msec and duration of 5 minutes. Each parameter was ramped in "real time" between maximum and minimum values at selected increments to see the effect of increasing the voltage on the product ion pair production. The spectra were parabola-like shape (disregarding individual spikes) with the value at the climax indicating the optimal setting for that parameter. Table 5 contains the setting of each parameter; entrance potential however was set at 10 based on the mass spectrometer manufacturer recommendations.

Table 5: Parameters setting to determine optimum values

Perameters	Range	Intervals
Collision Energy (CE)	5 – 150	1
Declustering Potential (DP)	5 – 80	0.2
Entrance Potential (EP)	10	
Collision Cell Entrance Potential (CEP)	5-50	0.1
Collision Cell Exit Potential (CXP)	0-5	0.1

The complete results of these three scans can be found in Table 6 and Table 7 however although these are the final values, the product ions (Q3) were changed in some cases. These changes were prompted by poor results in the later optimization steps causing the parameters to be revaluated to give the results provided in Table 6 and Table 7.

Table 6: Transitional pairs for each compound with optimized parameters

Mass Q1	Q3	Name	DP	EP	CEP	CE	CXP
427.3	207.3	9-hydroxyrisperidone 1	61	10	17	38	3
427.3	110.3	9-hydroxyrisperidone 2	61	10	22	58	3
431.3	211.3	9-hydroxyrisperidone-D4 1	61	10	19	38	3
431.3	114.3	9-hydroxyrisperidone-D4 2	61	10	20	58	2
448.3	176.3	Aripiprazole 1	64	10	20	41	3
448.3	98.3	Aripiprazole 2	64	10	20	52	3
456.3	293.3	Aripiprazole-D8 1	64	10	17	37	3
456.3	178.3	Aripiprazole-D8 2	64	10	17	45	3
386.3	122.3	Buspirone 1	68	10	16	42	3
386.3	95.3	Buspirone 2	68	10	22	75	3
394.3	122.3	Buspirone-D8 1	67	10	17	43	2
394.3	230	Buspirone-D8 2	63	10	40	38	3
319.3	58.3	Chlorpromazine 1	40	10	14	57	2
319.3	86.3	Chlorpromazine 2	40	10	13	29	3
322.3	61.3	Chlorpromazine-D3 1	41	10	14	60	3
322.3	89.3	Chlorpromazine-D3 2	41	10	13	29	3
327.3	270.3	Clozapine 1	51	10	32	30	3
327.3	192.3	Clozapine 2	51	10	18	58	3
331.3	272.3	Clozapine-D4 1	51	10	33	31	3
331.3	192.3	Clozapine-D4 2	51	10	20	59	3
438.3	171.3	Fluphenazine 1	60	10	16	35	3
438.3	143.3	Fluphenazine 2	60	10	23	39	3
446.3	151.3	Fluphenazine-D8 1	65	10	18	43	2
446.3	179.3	Fluphenazine-D8 2	62	10	40	34	2
376.3	123.3	Haloperidol 1	52	10	17	57	2
376.3	165.3	Haloperidol 2	52	10	15	32	3
380.3	169.3	Haloperidol-D4 1	53	10	14	33	2
380.3	127.3	Haloperidol-D4 2	53	10	19	55	2
328.3	271.3	Loxapine 1	50	10	34	29	3
328.3	193.3	Loxapine 2	50	10	20	59	3
336.3	276.3	Loxapine-D8 1	50	10	32	30	3
336.3	304.3	Loxapine-D8 2	50	10	39	32	3
387.3	98.3	Mesoridazine 1	55	10	17	56	2
387.3	126.3	Mesoridazine 2	55	10	15	34	2

Table 7: Transitional pairs for each compound with optimized parameters

Mass Q1	Q3	Name	DP	EP	CEP	CE	CXP
313.3	256.3	Olanzapine 1	55	10	32	29	3
313.3	213.3	Olanzapine 2	55	10	30	39	3
316.3	256.3	Olanzapine-D3 1	55	10	34	29	3
316.3	87.3	Olanzapine-D3 2	50	10	13	32	3
404.3	143.3	Perphenzine 1	55	10	15	39	3
404.3	171.3	Perphenzine 2	55	10	39	31	3
412.3	151.3	Perphenzine-D8 1	59	10	14	40	2
412.3	179.3	Perphenzine-D8 2	59	10	14	39	2
376.3	331.3	Pipamperone 1	48	10	45	25	3
376.3	138.3	Pipamperone 2	48	10	15	37	2
386.3	165.3	Pipamperone-D10 1	47	10	15	40	3
386.3	341.3	Pipamperone-D10 2	48	10	45	25	3
285.3	86.3	Promethazine 1	30	10	12	27	3
285.3	198.3	Promethazine 2	30	10	12	30	3
291.3	92.3	Promethazine-D6 1	32	10	10	26	3
291.3	77.3	Promethazine-D6 2	30	10	14	62	3
384.3	253.3	Quetiapine 1	63	10	39	30	3
384.3	221.3	Quetiapine 2	63	10	18	50	3
392.3	258.3	Quetiapine-D8 1	67	10	38	32	3
392.3	226	Quetiapine-D8 2	67	10	19	50	3
411.3	191.3	Risperidone 1	56	10	17	39	3
411.3	110.3	Risperidone 2	56	10	30	69	3
415.3	195.3	Risperidone-D4 1	55	10	20	39	3
415.3	114.3	Risperidone-D4 2	58	10	45	69	2
371.3	126.3	Thioridazine 1	49	10	14	31	2
371.3	98.3	Thioridazine 2	49	10	17	52	2
374.3	129.3	Thioridazine-D3 1	50	10	15	31	2
374.3	101.3	Thioridazine-D3 2	48	10	16	50	3
444.3	221.3	Thiothixene 1	57	10	30	70	3
444.3	70.3	Thiothixene 2	57	10	19	70	3
408.3	141.3	Trifluoperazine 1	53	10	16	32	3
408.3	70.3	Trifluoperazine 2	53	10	20	68	3
411.3	144.3	Trifluoperazine-D3 1	53	10	16	33	2
411.3	73.3	Trifluoperazine-D3 2	53	10	20	69	2
413.3	194.3	Ziprasidone 1	72	10	30	39	3
413.3	166.3	Ziprasidone 2	65	10	30	60	3
421.3	194.3	Ziprasidone-D8 1	65	10	16	39	3
421.3	182.3	Ziprasidone-D8 2	60	10	17	39	3

2.3 HPLC-MS Method Development

The next step was to determine retention times, so scans could be focused on ions in specific time windows to improve sensitivity. The HPLC eluents were prepared according to lab procedure and current literature for HPLC modes with antipsychotics [66] [50]. Mobile phase (A) was composed of 50mmol/L ammonium formate in water at pH 3.5. 3.15g of ammonium formate was added to 1000 mL of distilled water and pH was adjusted to 3.5 with formic acid. Mobile phase (B) was composed of 0.1% formic acid in acetonitrile. 100µL of formic acid was added to a 1000mL beaker which was then filled to the 1000ml mark with acetonitrile. During use, the mobile phase was degassed by the UFLC Shimadzu DGU-20A3 degasser. Stock solutions of the compounds were combined in methanol to make a concentration of 20µg/mL for each compound. 10µL of this compound mixture was completely dried down in the Zymark Turbovap LV (Caliper Life Sciences, Hopkinton, MA. USA) and reconstituted in 200µL of a mixture of mobile phase A and B (50:50) and transferred to an autosampler vial with an insert. A blank solution of a mixture of mobile phase A and B (50:50) is also put in an autosampler vial. Two blanks were then run prior to the sample in an unscheduled run (Scanning for all ions throughout the entire run, no time window). During this phase, parameters were developed by trial and error for the type of column, mobile phase solvents, oven temperature, flow rate (Table 8), and type of gradient elution (Table 9) to insure good separation, selectivity, sensitivity, and speed. The mobile phase solvents and the column listed above are a result of this section of the study.

Table 8: Parameters and setting of the Shimadzu HPLC

Shimadzu LC Method Parameters	
Equilibration time	5.00 min
Injection Volume	10.00 µL
Run Time	14.50 min
Pumps	
Pump A Motel: 1C-2QAD	
Pump B Motel: IC-20AD	
Pumping Mode	Binary Flow
Total Flow Rate	1.5 mL/min.
Pump B Pct	10%
B Curve	0
Pressure Range (Pump A/B)	0 - 300 Bars
Autosampler	
Model: SIL-2QSC	
Rinsing Volume	200 uL
Needle Stroke	50 mm
Rinsing Speed	35 µL/sec.
Sampling Speed	15.0 µL/sec.
Purge Time	25.0 min.
Rinse Dip Time	10 sec.
Rinse Mode	Before and after aspiration
Cooler Enabled	Yes
Cooler Temperature	15 deg. C
Control Vial Needle Stroke	52 mm
Oven	
Model: CTCH2CK	
Temperature	50 deg. C
Max.. Temperature	85 deg. C

Table 9: Gradient settings and ESI inlet parameters

Shimadzu LC Method Parameters				**AB Sceiex MS/MS**	
Time Program				**ESI-MS inlet parameters**	
Time	Module	Events	Parameter	CUR	30 psi
1.00	Pumps	Pump B Conc.	10	IS	5,500 V
12.00	Pumps	Pump B Conc.	60	TEM	700°C
12.50	Pumps	Pump B Conc.	10	GS1	65 psi
14.50	Systsn Controller	Stop		GS2	65 psi

Once these HPLC settings were established, the Ion spray voltage (IS), curtain gas (CUR) gas1 (GS1), gas 2 (GS2), and temperature of gas 2 (TEM) adjustments of the mass spectrometer values were set based on common laboratory practices in the lab and literature [67] [55] with final adjustment made to maximizing signal intensities and performance (Table 9). Adjustments were then made to designate which of the product ions would be primary (Ion 1) and secondary (Ion 2) based on intensity with the primary ion assigned to the higher intensity ion (Figure 20). The retention times were determined for all the analytes and internal standards (Figure 20) to schedule scans for each compound at the appropriate time with a 60 second window (30 seconds on each side of the retention time) to increase sensitivity by reducing the amount of scans throughout the run.

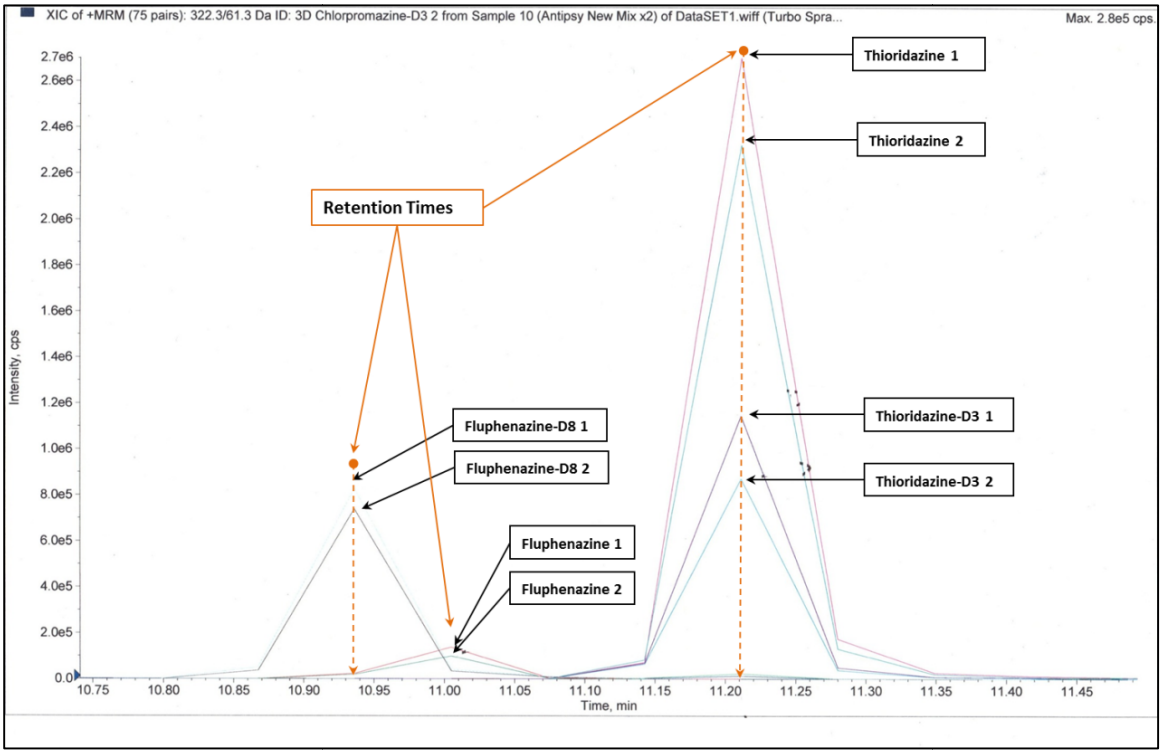


Figure 20: Determination of product ion position (intensity) and retention time. (This scan was taken during optimization so retention times in the figure are not accurate)

2.4 Method Validation

In an effort to validate this analytical method for analyzing the antipsychotic compounds, validation studies were performed to ensure reliability of the results. The parameters that were evaluated for quantitative assays of the antipsychotic compounds were: Determination of Linearity, Within and Between Day Accuracy and Precision, Carryover Evaluation, Matrix Effect and Recovery, and Peak Purity and Selectivity. Data

acquired from various sources (i.e. [17], Table 3) was used to compile information about therapeutic and lethal doses of all the 19 antipsychotic drugs. This information was used to generate a range in which to analyze these drugs. A stock solution of all of the analytes was prepared to a concentration of 5000 µg/L. Stock concentrations of the analytes were at concentrations of 1000 µg/mL, 100 µg/mL, and 40 µg/mL so 250 µL, 2.5 mL, and 6.25 mL were taken from each respectively and added to a 50 mL volumetric flask. The flask was then filled to the 50 mL mark on the flask with acetonitrile. A further dilution to 500µg/L was done by adding 2.5 mL of the first mixture to a 25 mL volumetric flask and filled to the 25 mL mark on the flask with acetonitrile. A stock solution of all of the internal standards were prepared to a concentration of 10 µg/L. Stock concentrations of the internal standards were at concentrations of 100 µg/mL and 10 µg/mL so 500 µL and 50 µL were taken from each respectively and added to a 10 mL volumetric flask. The flask was then filled to the 10 mL mark on the flask with acetonitrile. All stock solutions were stored in dark brown reaction vials wrapped in aluminum foil and stored at -70°C. 500 µL of blood, serum or urine is the amount always used that is spiked with the analytes and internal standard stocks. The blood concentration chosen were 10, 20, 50, 100, 400, 800, and 1000 µg/L. Table 10 contains the calculated amounts of analytes and internal standard to add for a desired blood concentration and also shows how many nano grams (ng) will be on the LC column.

Table 10: Calculated values determine the amount of analyte and IS needed to achieve selected blood concentrations

0.50mg/L = 0.5µg/mL = 0.5ng/µL = **500µg/L**						**Analye**	**IS**
(c)*(d) = (e)*(X)			**(X)*(e)*(1000) = h**			**Inject 10µL of c to get j on column**	
c(Blood Concentration)		**d**(Blood)	**e**(Stock Conc)	**X**	**h**		**j**
µg/L	µg/µL	µL	µg/µL	µL	ng		ng
10	1.0E-05	500.0	5.0E-04	**10**	5.0	Dry Down **X** and reconstitute with 100µL of Mobile phase (50:50) to get **c**	0.5
20	2.0E-05		5.0E-04	**20**	10.0		1
50	5.0E-05		5.0E-04	**50**	25.0		2.5
100	1.0E-04		5.0E-04	**100**	50.0		5
400	4.0E-04		5.0E-04	**400**	200.0		20
800	8.0E-04		5.0E-04	**800**	400.0		40
1000	1.0E-03		5.0E-04	**1000**	500.0		50
300	3.0E-04		5.00E-04	**300**	150.0		15

The calculated amount of analytes and internal standards were added to a test tube followed by 4mL of 0.1 mL phosphate buffer (pH 6.0) and vortexed briefly. 0.5mL of blood, serum, or urine was then added to the test tube vortex briefly, sonicated for 5 minutes and centrifuged for 15 minutes at 3600 rpm.

The samples were then loaded on to Clean Screen® 130mg 6 ml X-Cel 1 columns in the CEREX® System-48™ and eluted by gravity or at a rate of 1-2 mL/min (should be very slow rate). Columns were dried at max pressure for 5 minutes and rinsed with 2 mL of 0.1mL phosphate buffer (pH 6.0). All drying steps were performed with nitrogen. The columns were then washed with 1 mL of 2% acetic acid & 98% methanol and dried at maximum pressure for 15 minutes. The acid/neutral wash was done with 1 mL of a 50/50 mix of chloroform and acetone. Collection tubes were then placed under samples

and the analytes and internal standards were eluted with 2 mL of 2% ammonium hydroxide and ethyl acetate by gravity or at a rate of 1-2 ml/min (should be very slow rate). The samples were then evaporated to dryness with the Zymark TurboVap LV Evaporator® using nitrogen, reconstituted with 100 µL of a 50/50 mix of mobile phases (A) and (B) and transferred to autosampler vials. The autosampler vials were centrifuged at 3600 rpm for 4 minutes and the sample was ready for analysis.

2.4.1 Determination of Linearity

Following the procedures above six replicate samples were prepared in negative sheep's blood at concentrations of 10, 20, 50, 100, 400, 800, and 1000 µg/L (Table 10). Six replicates of the following samples (1-7) were prepared for a total of 48 extractions:

Sample 1. Add 10 µL of analyte stock. (Blood conc. 20 µg/L)
Sample 2. Add 20 µL of analyte stock. (Blood conc. 50 µg/L)
Sample 3. Add 50 µL of analyte stock. (Blood conc. 100 µg/L)
Sample 4. Add 100 µL of analyte stock. (Blood conc. 200 µg/L)
Sample 5. Add 400 µL of analyte stock. (Blood conc. 500 µg/L)
Sample 6. Add 800 µL of analyte stock. (Blood conc. 1000 µg/L)
Sample 7. Add 1000 µL of analyte stock. (Blood conc. 2000 µg/L)
Sample 8. No addition of analyte. (Blood conc. 0 µg/L)

Then to each sample 300 µL of the internal standard stock was added (Blood conc. 300 µg/L) followed by the addition of phosphate buffer and remaining steps above.

Using an Analyst® and Graph Pad® Prism software programs, calculations for precision and accuracy of the six replicates at each calibration point and the coefficient of determination (R^2) were determined.

2.4.2 Within and Between Day Accuracy and Precision

Samples were prepared in negative sheep's blood, serum and human urine at low, medium and high concentrations and analyzed in triplicate each day for a period of five days. The following samples were prepared for a total of 25 extractions per day:

New calibration curves were established with each run (7 extractions)

Sample 1. Add 10 µL of analyte stock. (Blood conc. 20 µg/L)
Sample 2. Add 20 µL of analyte stock. (Blood conc. 50 µg/L)
Sample 3. Add 50 µL of analyte stock. (Blood conc. 100 µg/L)
Sample 4. Add 100 µL of analyte stock. (Blood conc. 200 µg/L)
Sample 5. Add 400 µL of analyte stock. (Blood conc. 500 µg/L)
Sample 6. Add 800 µL of analyte stock. (Blood conc. 1000 µg/L)
Sample 7. Add 1000 µL of analyte stock. (Blood conc. 2000 µg/L)

Three replicates in blood and one of serum and one of urine (15 extractions)

Sample 8. Add 20 µL of analyte stock. (Blood conc. 50 µg/L)
Sample 9. Add 100 µL of analyte stock. (Blood conc. 200 µg/L)
Sample 10. Add 800 µL of analyte stock. (Blood conc. 1000 µg/L)

Negative samples (3 extractions)

Sample 11. Sheep Blood Negative (No IS)
Sample 12. Sheep Plasma Negative (No IS)
Sample 13. Human Urine Negative (No IS)

To each sample 300 µL of the internal standard stock was added (Blood conc. 300 µg/L) followed by the addition of phosphate buffer and remaining steps above.

The within-day and between accuracy and precision was calculated with the following equations:

% Accuracy = ((calculated conc. - target conc.) / target conc.) X 100 **(Equation 1)**

%. Precision = standard deviation / mean X 100 **(Equation 2)**

The measured mean concentration at each level must be within ± 20 % of the target concentration and the precision of the assay cannot exceed ± 20 % at each concentration used in the validation study for acceptable performance.

2.4.3 Carryover and Crosstalk Evaluation

A sample of the highest calibrators was prepared in sheep blood and injected five times, each time followed by a negative sheep blood sample. The following samples would be prepared according to procedure and run in this order five times.

Sample 1. Add 1000 µL of analyte stock. (Blood conc. 2000 µg/L) with IS (Blood conc. 300 µg/L)

Sample 2. 50/50 mix of mobile phases (Blank)

The analyte/IS abundance ratio should be 10% or less when compared to the highest calibrator.

Crosstalk was evaluated to determine if analytes or internal standards were being interpreted as other compounds in the study. This was done by spiking negative sheep's blood with each compound individually at a blood concentration of 2000 µg/L.

2.4.4 Matrix Effect and Recovery

A method considered useful in validation studies to determine the recovery and matrix effect is referred to as the Matuszewski approach [66] because it provides a quantitative estimation of matrix effects. This method was done with three different sets of six different blood samples (3*MEC, 2*MEC decomposed, 1*Pig/Sheep blood) for a total of 42 extractions.

The first set consists of the (Nonextracted standards) (6 replicates).

SET #1 NEAT (6 replicates) (No Extractions)

200 µL of analyte stock was evaporated to dryness, reconstitute with 100 µL of a 50/50 mobile phase (A) and (B) and transfer to autosampler vials.

Set two (Recovery Post Extracted Standards) is composed of negative bloods that were extracted (SPE) and then fortified with the analytes.

SET #2 RPES (3 replicates) (18 Extractions)

Procedure was followed except that after SPE was performed on the blood, the eluent was evaporated to dryness, reconstituted with 100 µL of a 50/50 mobile phase (A) and (B), 200 µL of analyte stock was added, and they were transferred to autosampler vials.

Set three (Recovery Extracted Standards) is composed of negative bloods that were fortified with the analytes and then extracted (SPE).

SET #3 RES (3 replicates) (18 Extractions)

According to procedure 200 µL of analyte stock was added to blood and SPE was performed. They were evaporated to dryness, reconstituted with 100 µL of a 50/50 mobile phase (A) and (B), and transferred to autosampler vials.

All 42 samples were injected and the absolute abundance for each analyte used in the following equations to determine matrix effect and recovery.

Matrix Effect (%) = (SET #2) / (SET #1) x 100 (**Equation 3**)

Recovery (%) = (SET #3) / (SET #2) x 100 (**Equation 4**)

2.4.5 Peak Purity and Selectivity

Nine negative blood samples from different negative medical examiner case (MEC) bloods (Human) and one sheep blood without analyte(s) or intended Internal

Standard (IS) were analyzed according to procedure to check for endogenous peaks that might interfere with the analytes or the IS. Two of the MEC blood samples were from decomposed bodies however, all sample were processed and analyzed the same.

Then according to procedure, one negative sheep blood plus IS only and one negative sheep blood containing the analytes and no IS was analyzed to check for the absence of analyte fragmentation ions in the IS peak or vice versa.

Sample 1. Add 400 µL of analyte stock (Blood conc. 500 µg/L) to negative blood.
Sample 2. Add 300 µL of the internal standard (Blood conc. 300 µg/L) to negative blood.

CHAPTER THREE: Results and Discussion

3.1 Method Development (Compound Optimization)

The individual acquisition parameters of the tandem mass spectrometer for the analytes as well as the internal standards are summarized in Table 11. The declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each drug and can be found in Table 9. Also, retention times of each analyte and internal standard used for quantification are compiled in Table 11. Chromatographic separations for each compound to further distinguish compounds with identical or similar molecular and fragmental masses are shown in Figure 21. All of these parameters and settings were entered in a method file in the Analyst® software and the method was validated.

Table 11: Retention times for all Analytes and Internal standards

Mass Q1	Q3	Time (min)	Name	Mass Q1	Q3	Time (min)	Name
427.3	207.3	6.6	9-hydroxyrisperidone 1	404.3	143.3	10.2	Perphenzine 1
427.3	110.3	6.6	9-hydroxyrisperidone 2	404.3	171.3	10.2	Perphenzine 2
431.3	211.3	6.6	9-hydroxyrisperidone-D4 1	412.3	151.3	10.2	Perphenzine-D8 1
431.3	114.3	6.6	9-hydroxyrisperidone-D4 2	412.3	179.3	10.2	Perphenzine-D8 2
448.3	176.3	9.6	Aripiprazole 1	376.3	331.3	6.2	Pipamperone 1
448.3	98.3	9.6	Aripiprazole 2	376.3	138.3	6.2	Pipamperone 2
456.3	293.3	9.5	Aripiprazole-D8 1	386.3	165.3	5.9	Pipamperone-D10 1
456.3	178.3	9.5	Aripiprazole-D8 2	386.3	341.3	5.9	Pipamperone-D10 2
386.3	122.3	7.4	Buspirone 1	285.3	86.3	8.9	Promethazine 1
386.3	95.3	7.4	Buspirone 2	285.3	198.3	8.9	Promethazine 2
394.3	122.3	7.4	Buspirone-D8 1	291.3	92.3	8.9	Promethazine-D6 1
394.3	230	7.4	Buspirone-D8 2	291.3	77.3	8.9	Promethazine-D6 2
319.3	58.3	10.2	Chlorpromazine 1	384.3	253.3	8.3	Quetiapine 1
319.3	86.3	10.2	Chlorpromazine 2	384.3	221.3	8.3	Quetiapine 2
322.3	61.3	10.2	Chlorpromazine-D3 1	392.3	258.3	8.3	Quetiapine-D8 1
322.3	89.3	10.2	Chlorpromazine-D3 2	392.3	226	8.3	Quetiapine-D8 2
327.3	270.3	8.2	Clozapine 1	411.3	191.3	7.2	Risperidone 1
327.3	192.3	8.2	Clozapine 2	411.3	110.3	7.2	Risperidone 2
331.3	272.3	8.1	Clozapine-D4 1	415.3	195.3	7.3	Risperidone-D4 1
331.3	192.3	8.1	Clozapine-D4 2	415.3	114.3	7.3	Risperidone-D4 2
438.3	171.3	11	Fluphenazine 1	371.3	126.3	11.2	Thioridazine 1
438.3	143.3	11	Fluphenazine 2	371.3	98.3	11.2	Thioridazine 2
446.3	151.3	10.9	Fluphenazine-D8 1	374.3	129.3	11.2	Thioridazine-D3 1
446.3	179.3	10.9	Fluphenazine-D8 2	374.3	101.3	11.2	Thioridazine-D3 2
376.3	123.3	8.9	Haloperidol 1	444.3	221.3	10	Thiothixene 1
376.3	165.3	8.9	Haloperidol 2	444.3	70.3	10	Thiothixene 2
380.3	169.3	8.8	Haloperidol-D4 1	408.3	141.3	11.7	Trifluoperazine 1
380.3	127.3	8.8	Haloperidol-D4 2	408.3	70.3	11.7	Trifluoperazine 2
328.3	271.3	9.1	Loxapine 1	411.3	144.3	11.7	Trifluoperazine-D3 1
328.3	193.3	9.1	Loxapine 2	411.3	73.3	11.7	Trifluoperazine-D3 2
336.3	276.3	9	Loxapine-D8 1	413.3	194.3	8	Ziprasidone 1
336.3	304.3	9	Loxapine-D8 2	413.3	166.3	8	Ziprasidone 2
313.3	256.3	4.5	Olanzapine 1	421.3	194.3	8	Ziprasidone-D8 1
313.3	213.3	4.5	Olanzapine 2	421.3	182.3	8	Ziprasidone-D8 2
316.3	256.3	4.5	Olanzapine-D3 1	387.3	98.3	8	Mesoridazine 1
316.3	87.3	4.5	Olanzapine-D3 2	387.3	126.3	8	Mesoridazine 2

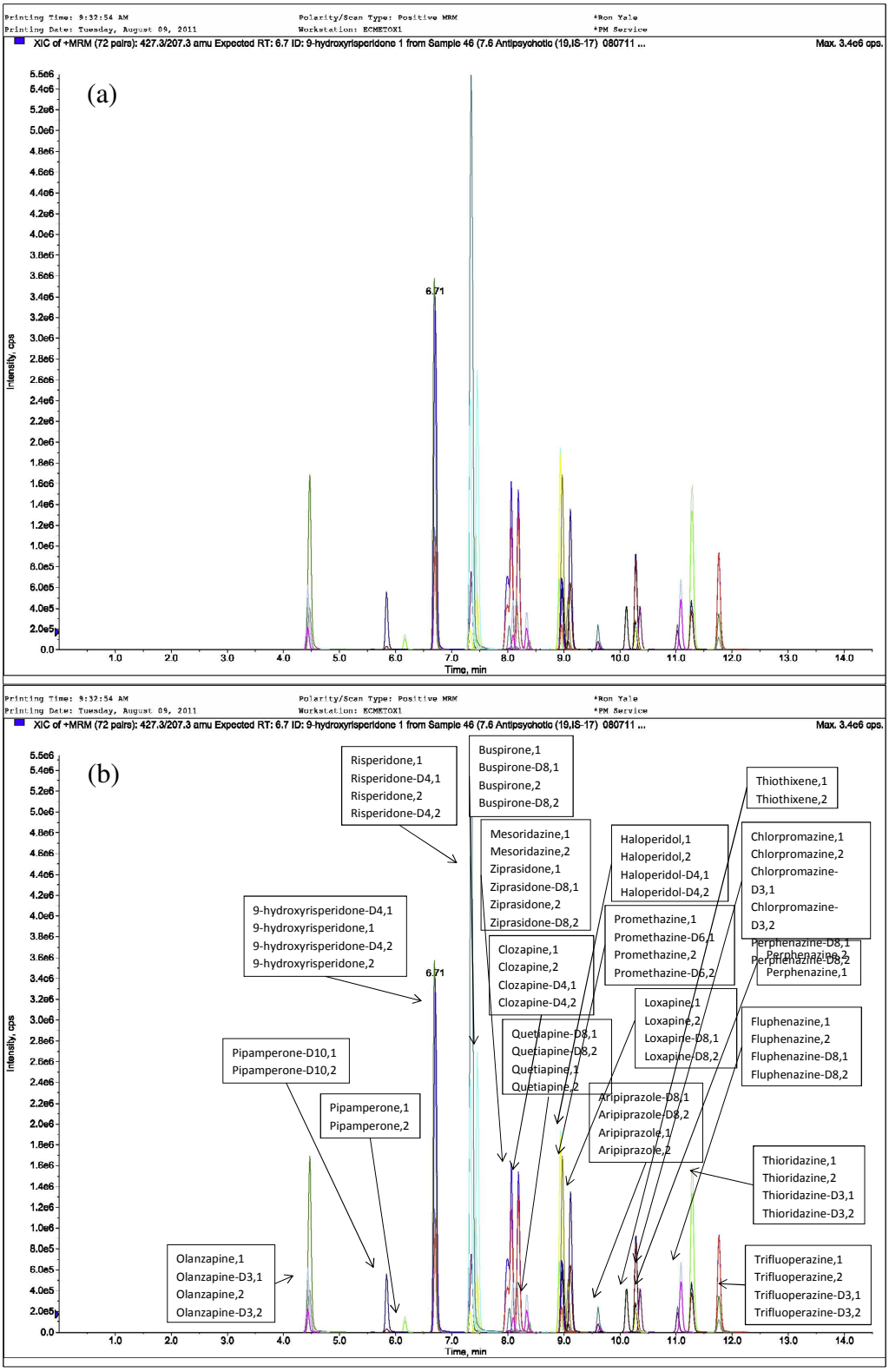


Figure 21: The extracted ion current chromatograms (XIC) of all analytes and internal standards (a) and labeled (b)

3.2 Method Validation

3.2.1 Determination of Linearity

Analyst® software performed the necessary calculations with the additional input of information such as peak integration, background range, curve smoothing, weighting factor (1/x for all compounds), and others to produce accurate data and curves. Transitioning between linear and quadratic fits produced an equation and a R^2 value to assist with determining the best model. Below Figure 22 contains both models of 9-hyroxyrisperidone and with a visual inspection and a R^2 (liner = 0.9958 quadratic = 0.9998) value it can be determined that this is second order (quadratic).

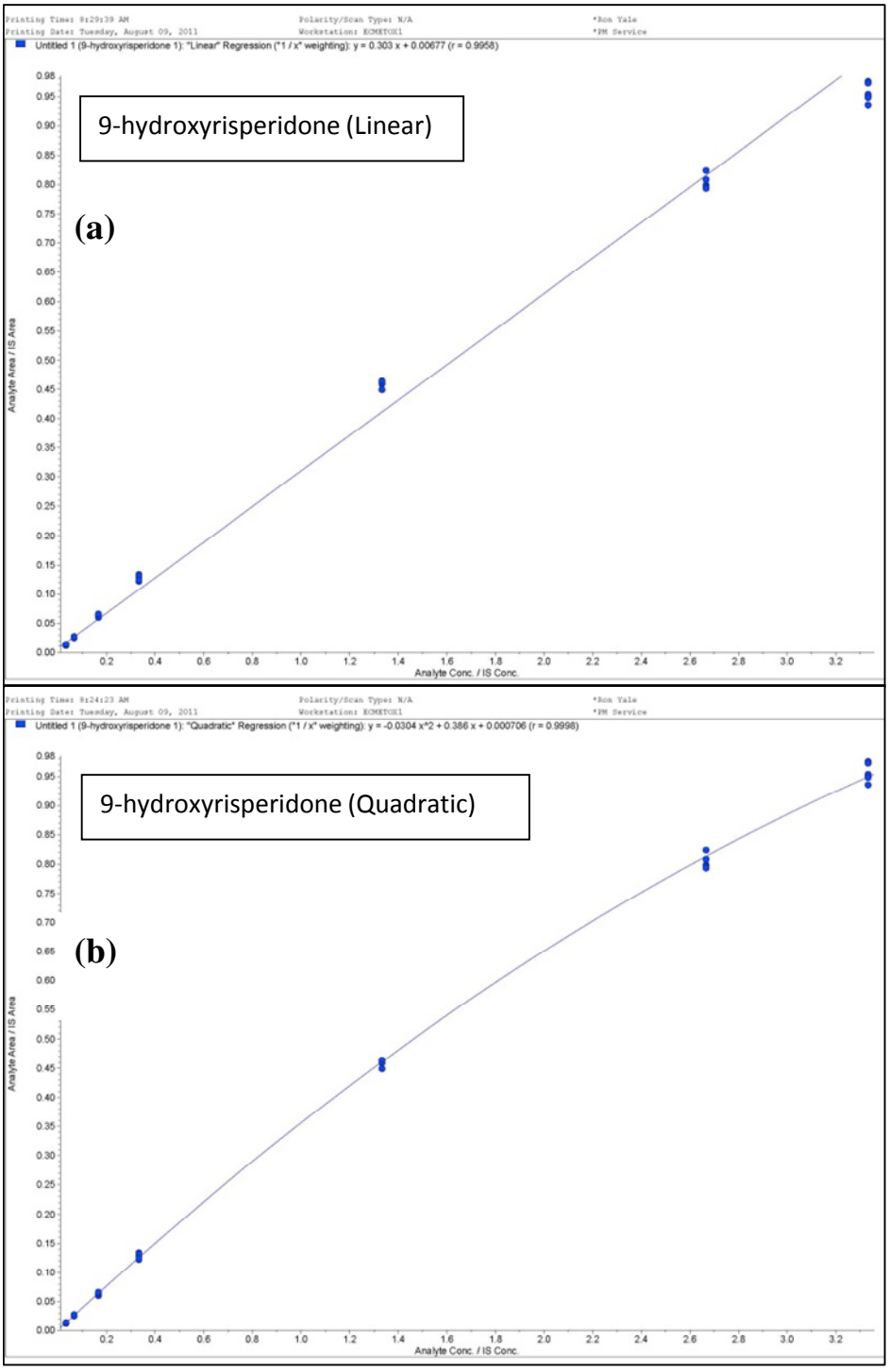


Figure 22: Liner (a) and quadratic (b) models for 9-hydroxyrispiradone (six replicates for each of seven concentrations)

To further verify these findings, the x (Analyte conc. /IS conc.) and y (Analyte area/IS area) values were put in the Graph Pad® Prism software program and provided information for all the analytes like that of 9-hydroxyrisperidone in Table 12. This provided further evidence of the model along with other valuable statistical information that can be used in support of the quantitation results of unknown samples. Table 13 contains a list of the antipsychotics and their predicted model. Analyst® charts and Prism data for each drug can be found in Appendix A.

Table 12: Graph Pad® Prism software out of quantitation data of 9-hydroxyrisperadone

9-hydroxyrisperidone	
Comparison of Fits	
Null hypothesis	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)
P value	< 0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis
Preferred model	Second order polynomial (quadratic)
F (DFn, DFd)	608.7 (1,38)
First order polynomial (straight line)	
Best-fit values	
B0	0.006731
B1	0.3036
Std. Error	
B0	0.001633
B1	0.004441
95% Confidence Intervals	
B0	0.003426 to 0.01003
B1	0.2946 to 0.3126
Goodness of Fit	
Degrees of Freedom	39
R square (weighted)	0.9917
Weighted Sum of Squares (1/X)	0.03067
Sy.x	0.02804
Second order polynomial (quadratic)	
Best-fit values	
B0	0.000711
B1	0.3854
B2	-0.03014
Std. Error	
B0	0.0004695
B1	0.003491
B2	0.001222
95% Confidence Intervals	
B0	-0.0002398 to 0.001662
B1	0.3783 to 0.3924
B2	-0.03262 to -0.02767
Goodness of Fit	
Degrees of Freedom	38
R square (weighted)	0.9995
Weighted Sum of Squares (1/X)	0.001802
Sy.x	0.006886
Number of points	
Analyzed	41

Table 13: List of drugs and their predicted quantification model

Compound	Preferred model
9-hydroxyrisperidone	Second order polynomial (quadratic)
Aripiprazole	First order polynomial (straight line)
Buspirone	Second order polynomial (quadratic)
Chlorpromazine	Second order polynomial (quadratic)
Clozapine	Second order polynomial (quadratic)
Fluphenazine	Second order polynomial (quadratic)
Haloperidol	Second order polynomial (quadratic)
Loxapine	Second order polynomial (quadratic)
Mesoridazine	Additional testing needed (quadratic)
Olanzapine	Second order polynomial (quadratic)
Perphenzine	First order polynomial (straight line)
Pipamperone	Second order polynomial (quadratic)
Promethazine	Second order polynomial (quadratic)
Quetiapine	First order polynomial (straight line)
Risperidone	Second order polynomial (quadratic)
Thioridazine	Second order polynomial (quadratic)
Thiothixene	Inconclusive
Trifluoperazine	Second order polynomial (quadratic)
Ziprasidone	Second order polynomial (quadratic)

In Table 13 mesoridazine is marked with “Additional testing need” because it was determined that thioridazine was being metabolized when mixed in blood. Figure 23 displays the lack of accuracy and precision of the points on the mesoridazine curves. This was due to the peak splitting seen below the curves, which was reduced when the entire peak was integrated but still introduced error.

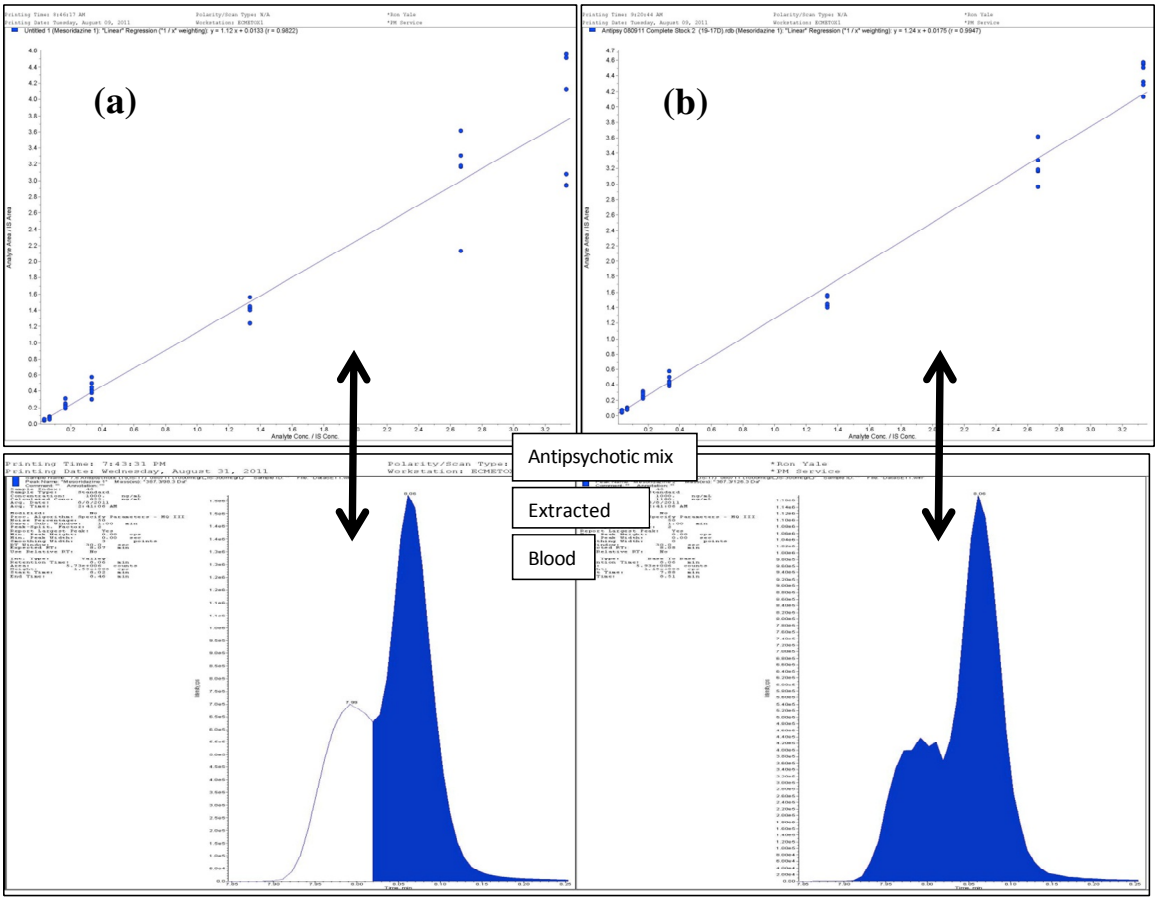


Figure 23: Mesoridazine curve with corresponding integrated peaks of a point showing spit peak behavior (a) and the effect of complete integration (b)

Analyzing pure mesoridazine however, did not show this peak splitting but when pure thioridazine was analyzed it showed both mesoridazine and thioridazine peaks. Thioridazine was the only compound, when studying crosstalk that an additional analyte of interest was detected. With increasing amounts of time, thioridazine was spiked and left in negative blood the mesoridazine peak increased and the thioridazine peak decreased (Figure 24). This result was quantified and was able to be repeated. As a result,

mesoridazine could not be analyzed in a mixture with thioridazine or thioridazine-D3. Other internal standards for mesoridazine were tested but a suitable replacement, which will provide the quality of results needed has not yet been found. A more complete picture of these results can be found in Appendix B.

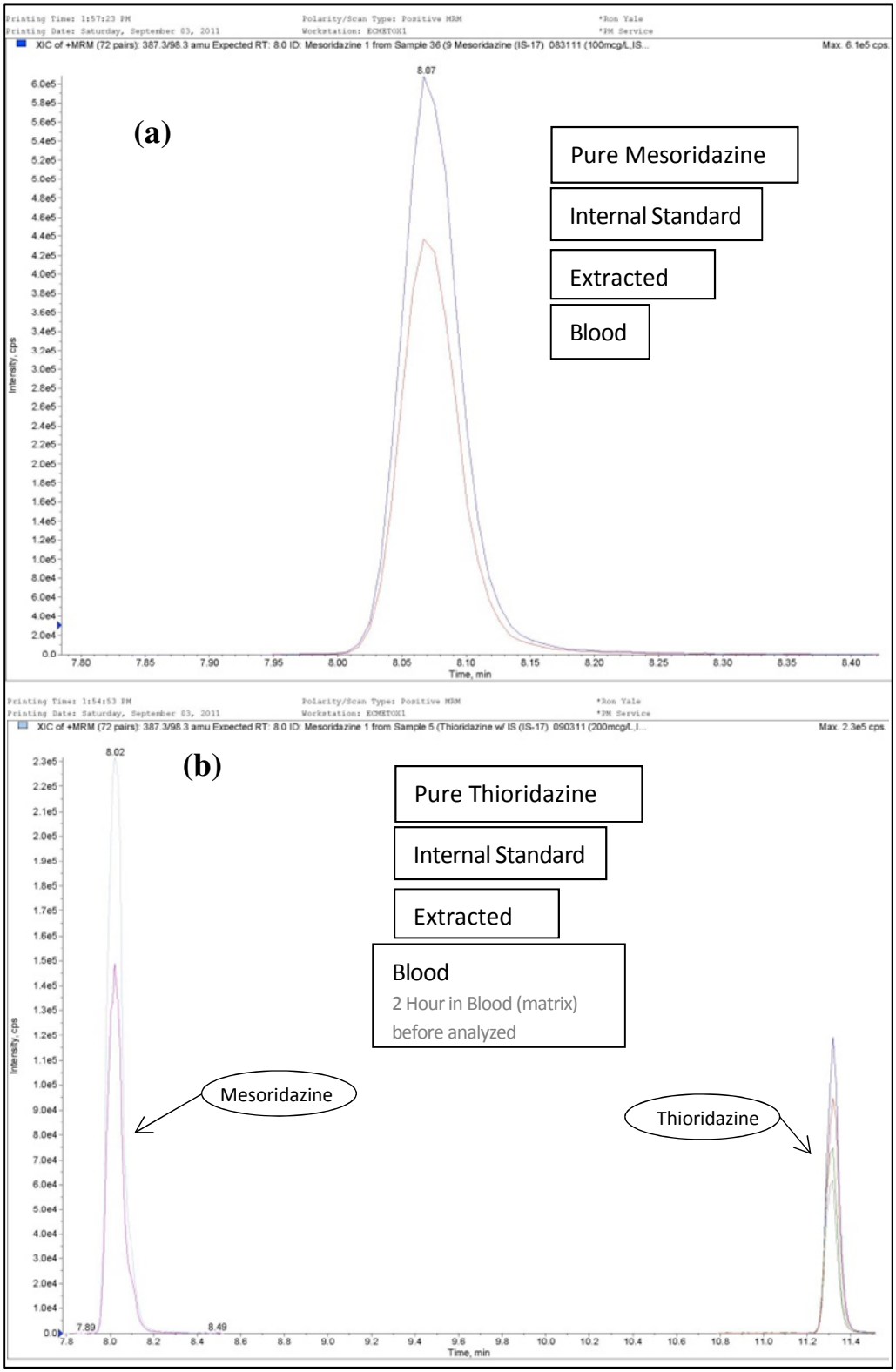


Figure 24: Pure mesoridazine (a) and pure thioridazine that has been in negative blood show a mesoridazine peak (b)

Thiothixene was the other compound that did not have its own deuterated internal standard and also did not produce results suitable enough to meet the guide lines for validation. However the data obtained can be used with the knowledge of its short comings until a more suitable internal standard is found.

3.2.2 Within and Between Day Accuracy and Precision

Although only five days was required for this validation, a total of eight days was used. During this study the HPLC column had a significant reduction in performance and was changed. This change was acceptable as it added a normal variation but additional days were run to ensure the new column was properly equilibrated and would provide consistent separations. Table 14 contains within and between day accuracy and precision for 9-hydroxrisperidone. The first column next to the headings blood, serum, and urine are the target concentrations in (µL) with three replicates of each for blood. The next column for each day is the calculated concentration followed by the % Accuracy (In Bold). For blood, this column is followed by calculations of the mean, standard deviation and precision. This is the within day precision and accuracy for each of the days shown. The between day precision is located in the bottom right corner with calculations for the mean, standard deviation and precision. These calculations are taken from all eight days and include precision data for serum and urine. Data for all compounds can be found in APPENDIX C

Table 14: Within & between day study results for 9-hydroxyrisperidone

9-hydroxyrisperidone																
Quadratic																
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	True Concentration	Measured Concentration	% Accuracy	Mean	SD	% Precision	Measured Concentration	% Accuracy	Mean	SD	% Precision	Measured Concentration	% Accuracy	Mean	SD	% Precision
Blood	20	21.8	9.00%	21.07	0.70	3.33%	21.2	6.00%	21.20	0.40	1.89%	18.8	6.00%	19.03	0.40	2.12%
	20	21	5.00%				21.6	8.00%				19.5	2.50%			
	20	20.4	2.00%				20.8	4.00%				18.8	6.00%			
	100	101	1.00%	104.33	3.06	2.93%	99.7	0.30%	100.43	4.25	4.23%	103	3.00%	101.43	3.61	3.56%
	100	105	5.00%				105	5.00%				104	4.00%			
	100	107	7.00%				96.6	3.40%				97.3	2.70%			
	800	807	0.88%	817.67	9.29	1.14%	782	2.25%	768.33	13.50	1.76%	796	0.50%	788.67	17.21	2.18%
	800	824	3.00%				755	5.63%				801	0.13%			
	800	822	2.75%				768	4.00%				769	3.88%			
Serum	20	21.1	5.50%				20.5	2.50%				19.5	2.50%			
	100	101	1.00%				105	5.00%				103	3.00%			
	800	818	2.25%				781	2.38%				763	4.63%			
Urine	20	21.1	5.50%				22.1	10.50%				18.5	7.50%			
	100	101	1.00%				96.9	3.10%				105	5.00%			
	800	805	0.63%				771	3.63%				775	3.13%			
		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.1	0.50%	19.00	1.10	5.79%	19.6	2.00%	19.73	0.15	0.77%	20.5	2.50%	20.03	0.90	4.47%
	20	17.9	10.50%				19.7	1.50%				20.6	3.00%			
	20	19	5.00%				19.9	0.50%				19	5.00%			
	100	93.9	6.10%	95.40	1.50	1.57%	104	4.00%	100.33	3.33	3.32%	101	1.00%	99.07	3.35	3.38%
	100	95.4	4.60%				97.5	2.50%				95.2	4.80%			
	100	96.9	3.10%				99.5	0.50%				101	1.00%			
	800	781	2.38%	768.67	16.44	2.14%	771	3.63%	773.67	24.11	3.12%	798	0.25%	775.33	23.01	2.97%
	800	775	3.13%				751	6.13%				752	6.00%			
	800	750	6.25%				799	0.13%				776	3.00%			
Serum	20	19	5.00%				21.5	7.50%				20.5	2.50%			
	100	98.5	1.50%				107	7.00%				99	1.00%			
	800	754	5.75%				765	4.38%				804	0.50%			
Urine	20	19.2	4.00%				18.7	6.50%				18.5	7.50%			
	100	92.5	7.50%				111	11.00%				98.3	1.70%			
	800	740	7.50%				787	1.63%				765	4.38%			
		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix							Between Day		
Blood	20	20.4	2.00%	20.37	0.06	0.28%	21.1	5.50%	21.35	0.35	1.66%			20.17	1.02	5.06%
	20	20.4	2.00%													
	20	20.3	1.50%				21.6	8.00%								
	100	102	2.00%	106.33	3.79	3.56%	107	7.00%	106.67	1.53	1.43%			101.75	4.53	4.46%
	100	108	8.00%				108	8.00%								
	100	109	9.00%				105	5.00%								
	800	800	0.00%	801.33	17.04	2.13%	812	1.50%	820.00	23.07	2.81%			789.21	25.65	3.25%
	800	819	2.38%				802	0.25%								
	800	785	1.88%				846	5.75%								
Serum	20	19.9	0.50%				21.3	6.50%						20.41	0.89	4.36%
	100	109	9.00%				112	12.00%						104.31	4.83	4.63%
	800	809	1.13%				814	1.75%						788.50	25.72	3.26%
Urine	20	19.5	2.50%				19.8	1.00%						19.68	1.30	6.63%
	100	116	16.00%				110	10.00%						103.84	8.05	7.76%
	800	797	0.38%				804	0.50%						780.50	22.26	2.85%

3.2.3 Carryover Evaluation

The carryover evaluation generated positive results. Analytes and IS showing peaks ranging between 1.0e5 to 6.0e6 cps and blank samples that followed each only showed maximums of up to 2186 cps or lower. This was significantly below the lower level of detection as previously defined meeting acceptable criteria for validation. Figure 25 shows the ion chromatograms of the injected analytes and IS (a) followed by the blank (b), the other chromatograms of the blanks can be found in. APPENDIX D (Preceding analyte chromatograms not shown).

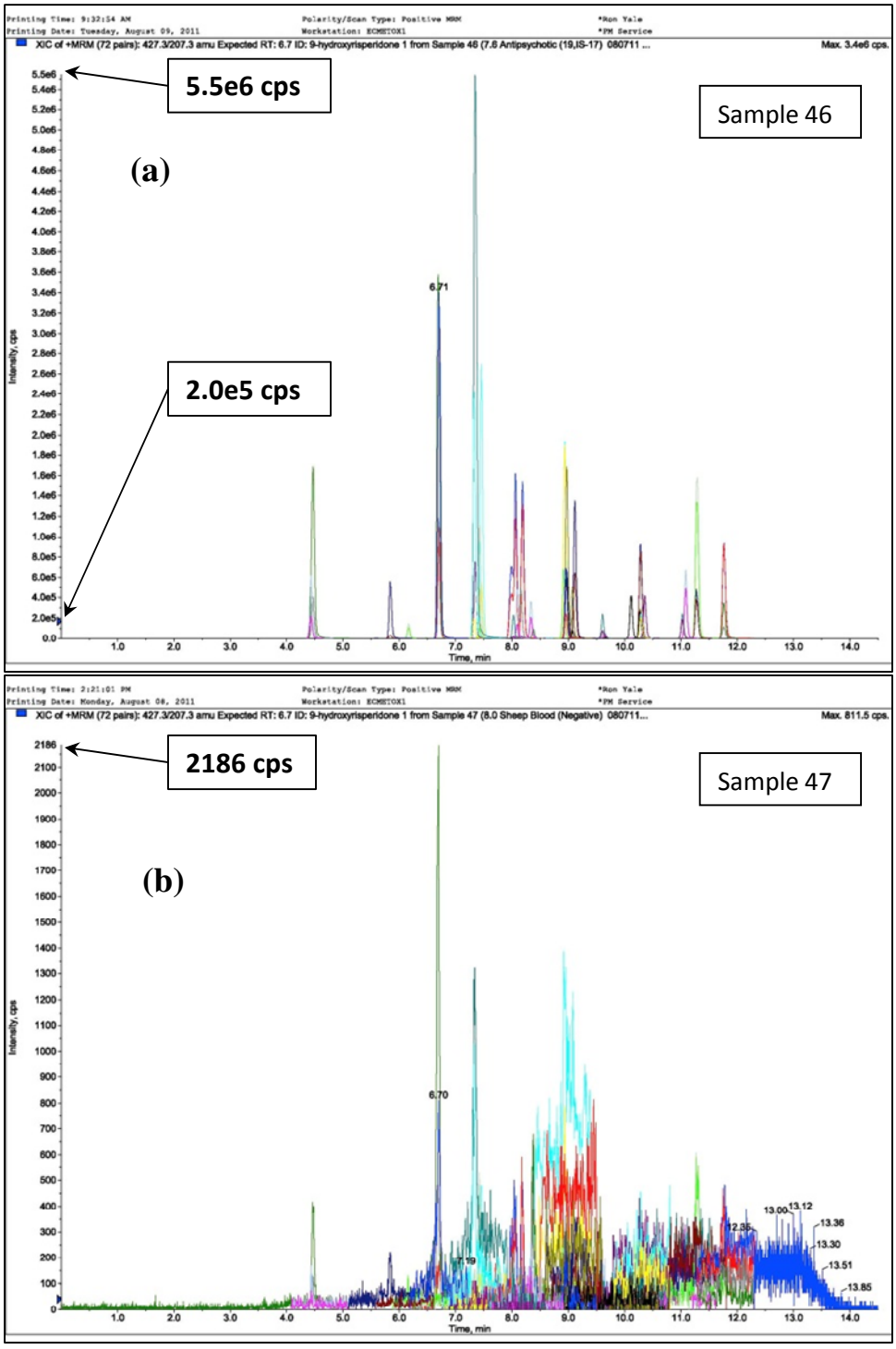


Figure 25: Carryover study, Sample 46 is the chromatogram of the analytes & IS injected (a) followed by a blank (b)

3.2.4 Matrix Effect and Recovery

Table 15 contains the mean values and ranges of recoveries and matrix effects of each analyte. The matrix effects and recoveries of most of the analytes exceed 80% when examining the differences between the mean and the range. Only perphenazine had a range difference much greater than ±20% of the mean value (Table 15 in red), however, when employing deuterated internal standard, it is assumed that the deuterated compound will be affected the same as the compound itself. This, however, would have a very large impact on compounds not using a deuterated internal standard, nevertheless all other compounds produced acceptable results. Mesoridazine and promethazine had values that were slightly above ±20% (Table 15 in red), however there were other validation issues with mesoridazine and promethazine like perphenazine, also uses a deuterated internal standard. Therefore, a large variation in range should have very little impact on the quantification of perphenazine. Olanzapine is known to be an exceptionally unstable drug and these results where no exception [54]. Olanzapine had very poor recoveries and matrix effects but it also has its own deuterated internal standard. Detailed recoveries and matrix effects of each analyte with the different negative bloods can be found in APPENDIX E.

Table 15: Mean values and ranges of recoveries and matrix effects. Values in red were greater than ±20% of the mean value

Compound	Parameter	Mean	Range	
9-hydroxyrisperidone	Matrix Effect	**105.17%**	103.39%	106.22%
	Recovery	**71.09%**	67.24%	75.51%
Aripiprazole	Matrix Effect	**102.93%**	101.27%	107.71%
	Recovery	**70.14%**	61.90%	74.94%
Buspirone	Matrix Effect	**107.35%**	106.41%	108.06%
	Recovery	**77.72%**	73.00%	80.93%
Chlorpromazine	Matrix Effect	**102.91%**	98.84%	105.24%
	Recovery	**60.16%**	54.04%	65.70%
Clozapine	Matrix Effect	**103.99%**	101.63%	107.44%
	Recovery	**76.27%**	73.25%	79.78%
Fluphenazine	Matrix Effect	**101.78%**	98.65%	104.27%
	Recovery	**53.03%**	45.54%	59.54%
Haloperidol	Matrix Effect	**104.86%**	102.46%	107.40%
	Recovery	**73.39%**	68.06%	78.07%
Loxapine	Matrix Effect	**108.15%**	105.84%	110.51%
	Recovery	**73.97%**	67.46%	79.65%
Mesoridazine	Matrix Effect	**103.92%**	101.97%	107.02%
	Recovery	**54.72%**	47.14%	66.02%
Olanzapine	Matrix Effect	**94.38%**	89.82%	97.96%
	Recovery	**15.61%**	13.12%	17.53%
Perphenzine	Matrix Effect	**49.15%**	23.94%	101.62%
	Recovery	**71.58%**	24.56%	177.36%
Pipamperone	Matrix Effect	**104.36%**	97.54%	109.06%
	Recovery	**75.29%**	72.70%	77.95%
Promethazine	Matrix Effect	**105.74%**	103.09%	107.88%
	Recovery	**69.93%**	55.95%	87.25%
Quetiapine	Matrix Effect	**103.68%**	100.47%	106.09%
	Recovery	**76.65%**	69.31%	80.96%
Risperidone	Matrix Effect	**106.86%**	105.69%	107.97%
	Recovery	**71.89%**	66.42%	79.85%
Thiotixene	Matrix Effect	**105.01%**	102.21%	107.30%
	Recovery	**60.09%**	52.67%	70.69%
Thioridazine	Matrix Effect	**101.19%**	97.77%	104.59%
	Recovery	**57.27%**	52.29%	61.71%
Trifluoperazine	Matrix Effect	**96.55%**	87.50%	100.34%
	Recovery	**53.70%**	47.17%	58.60%
Ziprasidone	Matrix Effect	**109.89%**	105.40%	111.70%
	Recovery	**67.16%**	56.37%	74.09%

3.2.5 Peak Purity and Selectivity

Ten negative specimens of the same matrix from different cases without analyte(s) or intended Internal Standard (IS) were analyzed to check for endogenous peaks that might interfere with the analyte or the IS. The analysis yielded no peaks above 1700 cps which was significantly below the lower level of detection as previously defined. Figure 26 shows the ion chromatograms of an injected negative blood sample. Ion chromatograms of the other ten samples can be found in APPENDIX F.

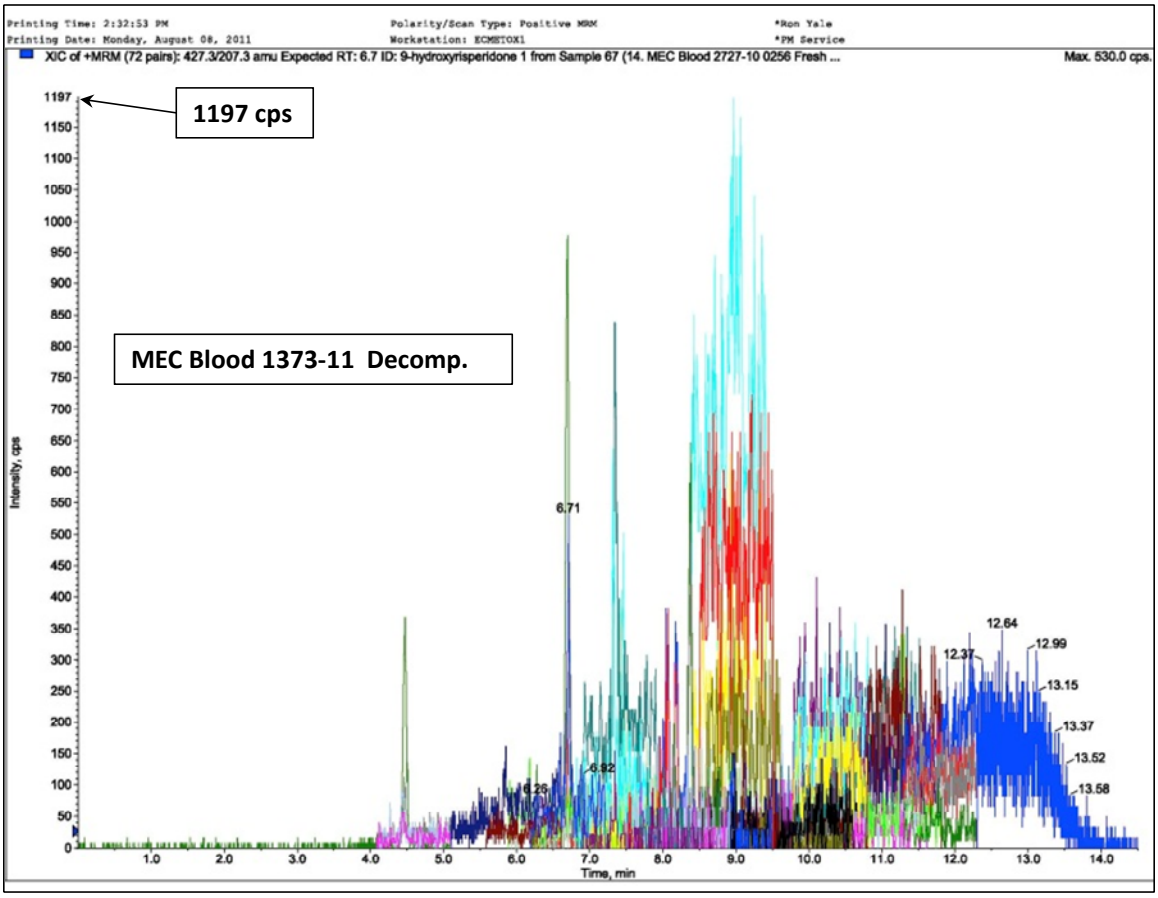


Figure 26: Peak Purity and Selectivity: Chromatogram of one of the ten different samples of negative blood

When analyzing negative specimens with IS or analytes, they showed no significant amounts of the other. So IS shows no analyte peaks and analytes show no IS peaks, therefore confirming the absence of analyte fragmentation ions in the IS peaks and vice versa. Figure 27 shows chromatograms for two samples with IS only (2A) and analytes only (1A), and that same chromatogram showing the amount of the opposing compound (i.e. IS or analytes (1&2B)).

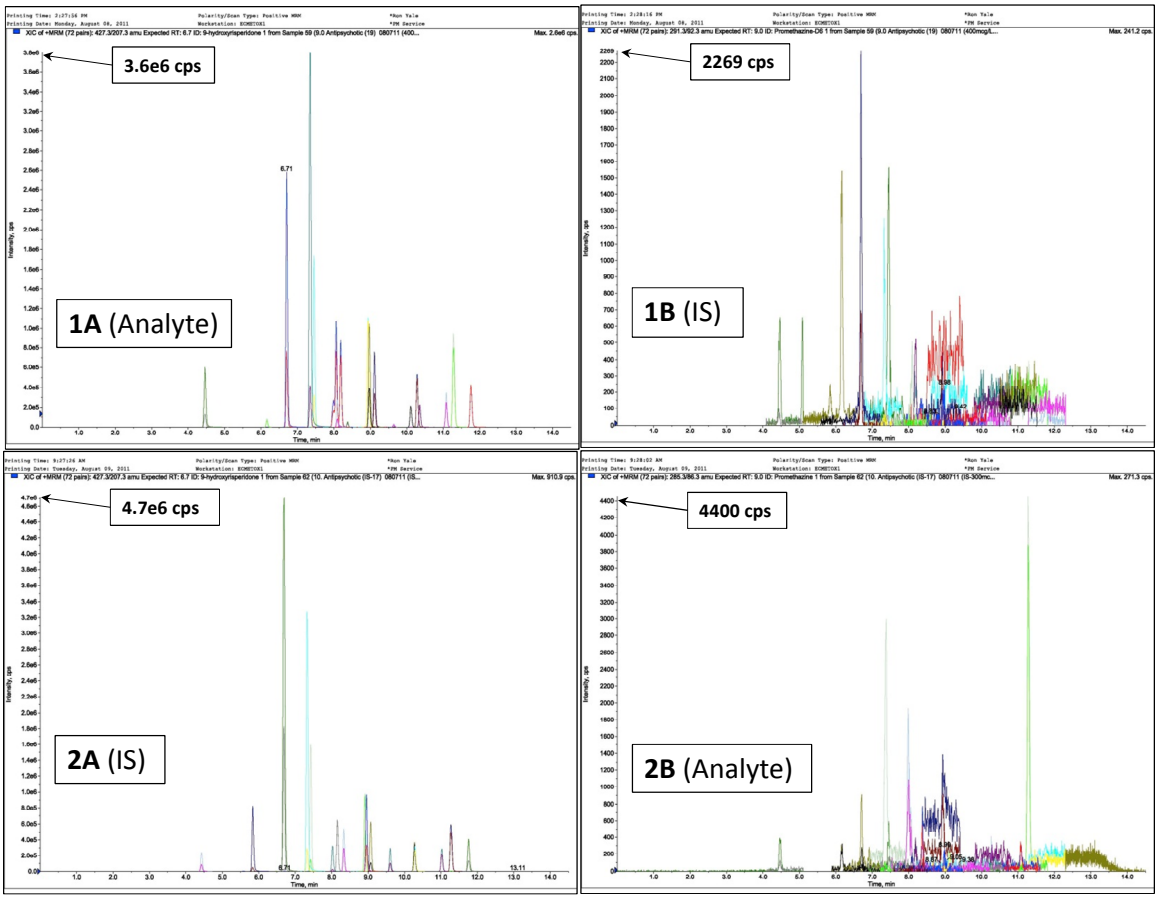


Figure 27: Sample 1 & 2 show one chromatograms each just looking at the IS or analytes. Sample 2 is IS only and sample 1 is analytes only.

Table 16 contains the analyte and IS peak area counts of the chromatogram (1) shown in Figure 27 for each sample showing the values of each of the peaks.

Table 16: Area counts of 1 A & B (**Figure 27**) showing the large range between IS & analytes within the same sample.

Sample 62			
Analyte Peak Name	**Analyte Peak Area (counts)**	**IS Peak Area (counts)**	**Calculated Concentration (ng/mL)**
9-hydroxyrisperidone 1	3.60E+03	1.76E+07	< 0
Aripiprazole 1	1.73E+02	9.36E+05	0.192
Buspirone 1	1.53E+03	5.22E+06	< 0
Chlorpromazine 1	7.37E+02	1.26E+06	0.0722
Clozapine 1	1.24E+03	2.14E+06	< 0
Fluphenazine 1	8.91E+02	1.03E+06	< 0
Haloperidol 1	1.81E+03	3.00E+06	< 0
Loxapine 1	8.02E+02	2.16E+06	< 0
Mesoridazine 1	9.68E+03	2.45E+06	< 0
Olanzapine 1	1.52E+03	8.94E+05	1.1
Perphenzine 1	3.01E+02	1.10E+06	0.261
Pipamperone 1	1.03E+03	2.67E+06	< 0
Promethazine 1	7.71E+02	3.23E+06	0.0321
Quetiapine 1	2.56E+03	1.73E+06	0.86
Risperidone 1	1.14E+04	1.18E+07	< 0
Thioridazine 1	1.73E+04	2.45E+06	< 0
Thiotixene 1	1.55E+02	1.03E+06	< 0
Trifluoperazine 1	2.38E+02	1.56E+06	0.00549
Ziprasidone 1	4.00E+02	1.09E+06	0.595

3.4 Nitrogen vs. Air

Originally the SPE and all other drying processes were performed using air, however, poor results led to the possibility of chemical reactions such as sulfoxide formation, changing the compounds. Many of these compounds contained sulfur and the introduction of oxygen in the air and at many times with elevated temperatures, which could cause a reaction changing the structure and molecular weight to produce a new undetected compound. This was tested by using nitrogen instead of air and the effects were compared in the matrix study. The results can be viewed in Table 17 which shows improvements all analyte in terms of recovery and matrix effect in the column labeled % increase with nitrogen. Only two compounds appear to be partially improved with the use of air, but further examination will show this is because of the large range in the data reducing the accuracy and precision of these results. The range of the data about the mean with air was much greater then with nitrogen for all compounds, although only those exceeding ±20% are labeled in red.

Table 17: Matrix study comparison of air vs. nitrogen for each analyte. . Values in red were greater than ±20% of the mean value

Compound	Parameter	Nitrogen			Air			% Increase with Nitrogen
		Mean	Range		Mean	Range		
9-hydroxyrisperidone	Matrix Effect	105.17%	103.39%	106.22%	87.96%	81.55%	99.39%	16.36%
	Recovery	71.09%	67.24%	75.51%	46.90%	42.39%	51.37%	34.03%
Aripiprazole	Matrix Effect	102.93%	101.27%	107.71%	89.50%	79.74%	102.56%	13.04%
	Recovery	70.14%	61.90%	74.94%	44.36%	38.84%	49.95%	36.76%
Buspirone	Matrix Effect	107.35%	106.41%	108.06%	85.52%	75.73%	98.92%	20.33%
	Recovery	77.72%	73.00%	80.93%	51.92%	46.04%	62.32%	33.19%
Chlorpromazine	Matrix Effect	102.91%	98.84%	105.24%	73.79%	66.46%	84.72%	28.29%
	Recovery	60.16%	54.04%	65.70%	29.37%	24.05%	33.06%	51.18%
Clozapine	Matrix Effect	103.99%	101.63%	107.44%	84.70%	78.17%	97.96%	18.55%
	Recovery	76.27%	73.25%	79.78%	44.29%	38.01%	52.53%	41.93%
Fluphenazine	Matrix Effect	101.78%	98.65%	104.27%	71.88%	63.45%	80.72%	29.38%
	Recovery	53.03%	45.54%	59.54%	27.09%	21.51%	38.14%	48.93%
Haloperidol	Matrix Effect	104.86%	102.46%	107.40%	86.98%	76.26%	99.46%	17.05%
	Recovery	73.39%	68.06%	78.07%	49.67%	45.01%	58.03%	32.32%
Loxapine	Matrix Effect	108.15%	105.84%	110.51%	87.53%	76.95%	101.00%	19.07%
	Recovery	73.97%	67.46%	79.65%	48.37%	43.67%	57.16%	34.61%
Mesoridazine	Matrix Effect	103.92%	101.97%	107.02%	92.86%	77.65%	114.33%	10.64%
	Recovery	54.72%	47.14%	66.02%	23.65%	17.41%	30.58%	56.78%
Olanzapine	Matrix Effect	94.40%	89.82%	97.96%	3.65%	0.86%	6.55%	96.13%
	Recovery	15.61%	13.12%	17.53%	17.44%	4.53%	33.43%	-11.71%
Perphenzine	Matrix Effect	49.15%	23.94%	101.62%	64.34%	57.38%	74.89%	-30.91%
	Recovery	71.58%	24.56%	177.36%	20.25%	15.68%	24.31%	71.71%
Pipamperone	Matrix Effect	104.36%	97.54%	109.06%				
	Recovery	75.29%	72.70%	77.95%				
Promethazine	Matrix Effect	105.74%	103.09%	107.88%	78.17%	70.03%	93.69%	26.08%
	Recovery	69.93%	55.95%	87.25%	31.41%	24.42%	33.94%	55.08%
Quetiapine	Matrix Effect	103.68%	100.47%	106.09%	89.00%	81.41%	101.44%	14.16%
	Recovery	76.65%	69.31%	80.96%	52.05%	47.27%	59.51%	32.09%
Risperidone	Matrix Effect	106.86%	105.69%	107.97%	82.32%	72.91%	98.15%	22.96%
	Recovery	71.89%	66.42%	79.85%	35.79%	29.65%	42.24%	50.21%
Thiothixene	Matrix Effect	105.01%	102.21%	107.30%	84.60%	73.61%	96.59%	19.44%
	Recovery	60.09%	52.67%	70.69%	29.91%	25.85%	34.23%	50.23%
Thioridazine	Matrix Effect	101.19%	97.77%	104.59%	69.17%	62.93%	78.46%	31.64%
	Recovery	57.27%	52.29%	61.71%	21.36%	16.36%	24.68%	62.71%
Trifluoperazine	Matrix Effect	96.55%	87.50%	100.34%	65.95%	55.96%	79.84%	31.70%
	Recovery	53.70%	47.17%	58.60%	24.69%	19.92%	29.91%	54.03%
Ziprasidone	Matrix Effect	109.89%	105.40%	111.70%	86.77%	78.09%	100.60%	21.04%
	Recovery	67.16%	56.37%	74.09%	47.80%	43.00%	53.34%	28.83%

CHAPTER FOUR: Summary and Conclusions

The LC-MS/MS assay developed is a suitable analytical method for the simultaneous separation, detection and quantification of 18 antipsychotics in postmortem human blood. The selectivity, linearity, accuracy and precision of the method were found to be suitable for forensic toxicological analyses. Promethazine had two values that were slightly above ±20%. Perphenazine had a range difference much greater than ±20% of the mean value, which could be considered an unacceptable large variation within the matrix study. However, the use of deuterated perphenazine and promethazine as an internal standard would nullify this effect assuming that the deuterated compound is affected in the same way as the compound itself. Through each phase of the validation all but two antipsychotics met the acceptable criteria for full validation. Mesoridazine and thiothixene require further study to find a more suitable IS to improve their results in linearity, accuracy and precision; however, thiothixene can still be evaluated with the knowledge of its limitations in this method. Recent studies into the use of ascorbic acid as an antioxidant to improve stability may improve the detection of thiothixene as well as other compounds in this study [67]. Because thioridazine is metabolizing to mesoridazine in post-mortem blood very quickly, thioridazine and mesoridazine cannot be evaluated in the same assay. This also brings up the challenge of postmortem analysis because thioridazine represents one of possibly many of these drugs that is metabolizing in blood making the concentration of drug no longer what it was at the time of death. The use of nitrogen as opposed to air for sample drying and SPE resulted in very clean extracts with good recoveries which had an average increase in recoveries by more the 45% and an

increase in the value of matrix effect by more than 25%. The presented LC-MS/MS assay has been found to be a simple and sensitive procedure that is suitable for use in forensic toxicology investigations.

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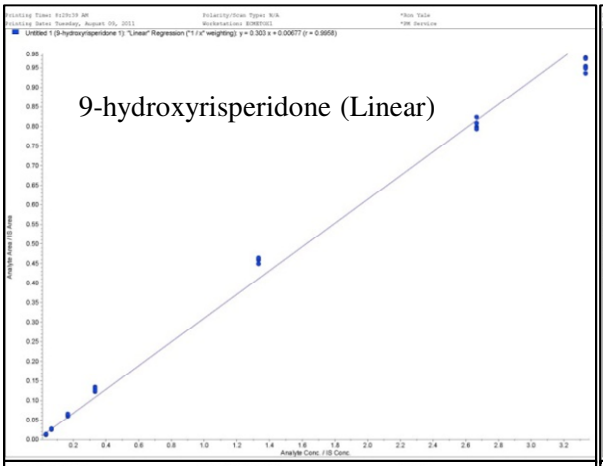
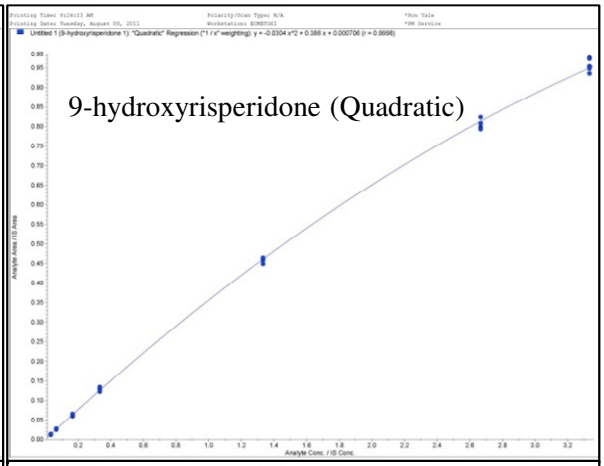
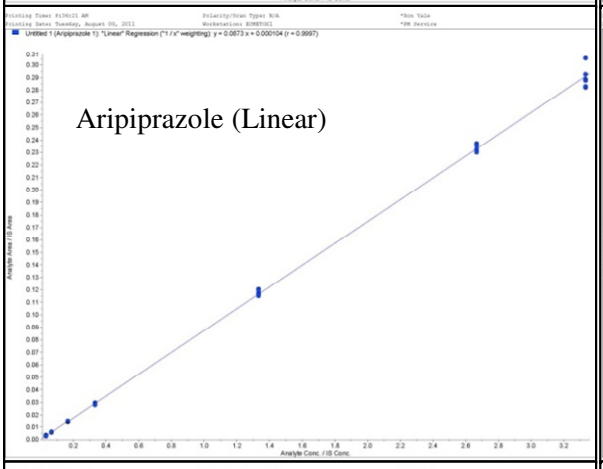
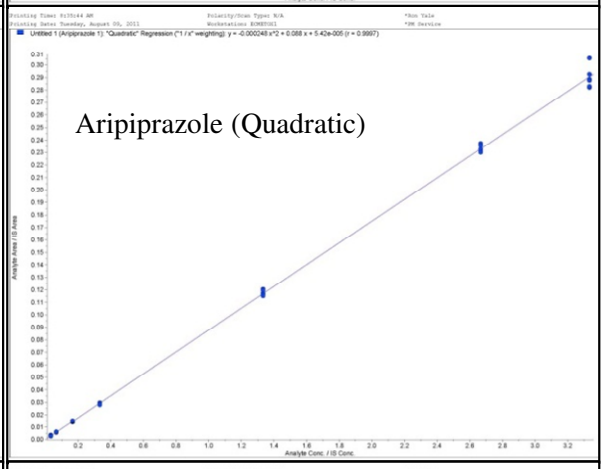
APPENDIX A

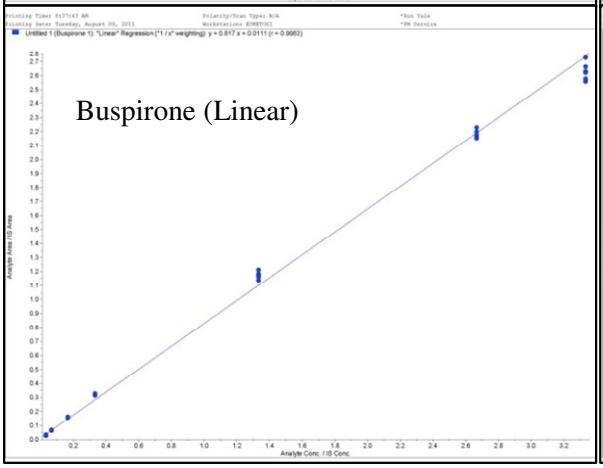
Analyst® Software Calibration Model Data

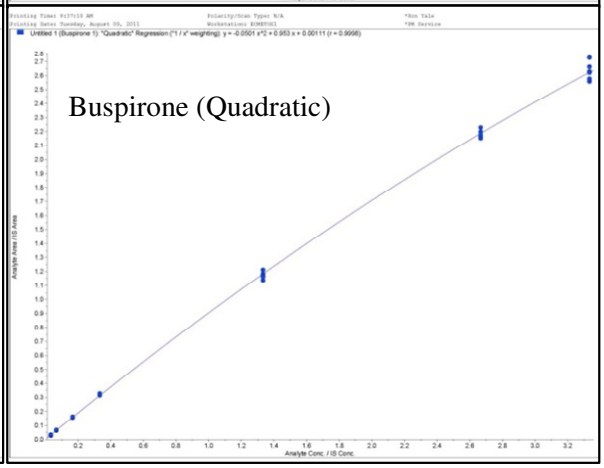


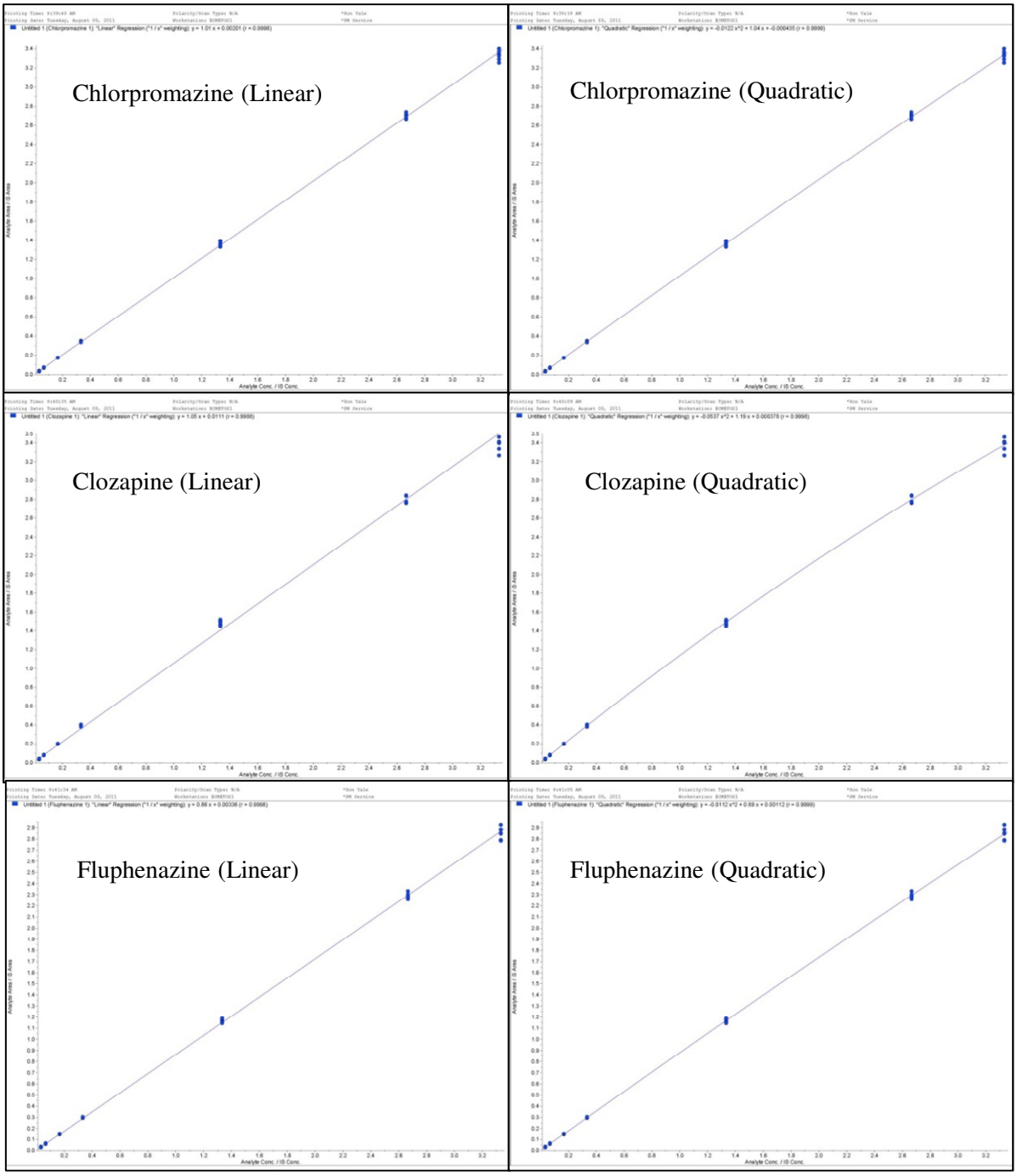


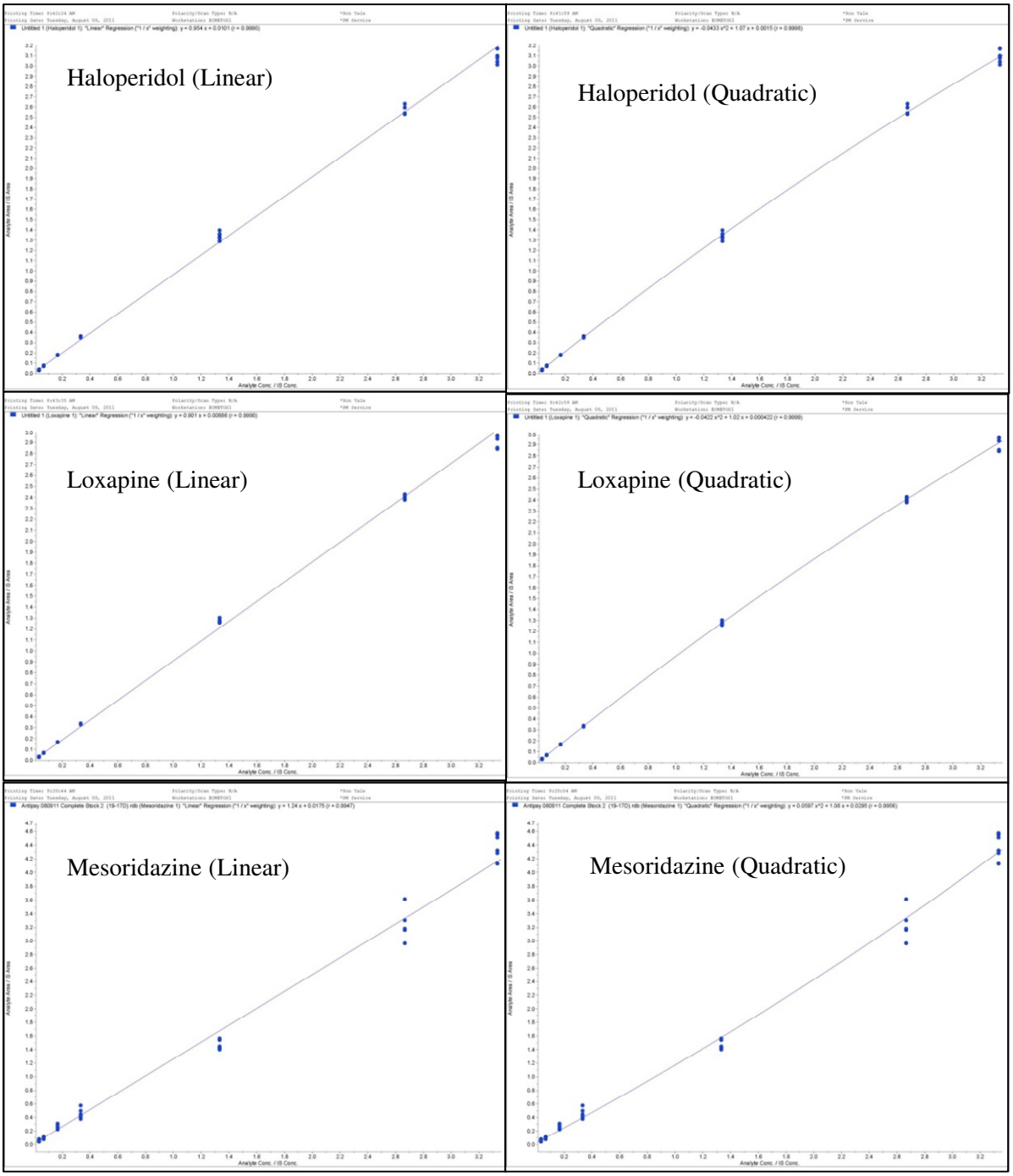









Chlorpromazine (Linear)
Chlorpromazine (Quadratic)
Clozapine (Linear)
Clozapine (Quadratic)
Fluphenazine (Linear)
Fluphenazine (Quadratic)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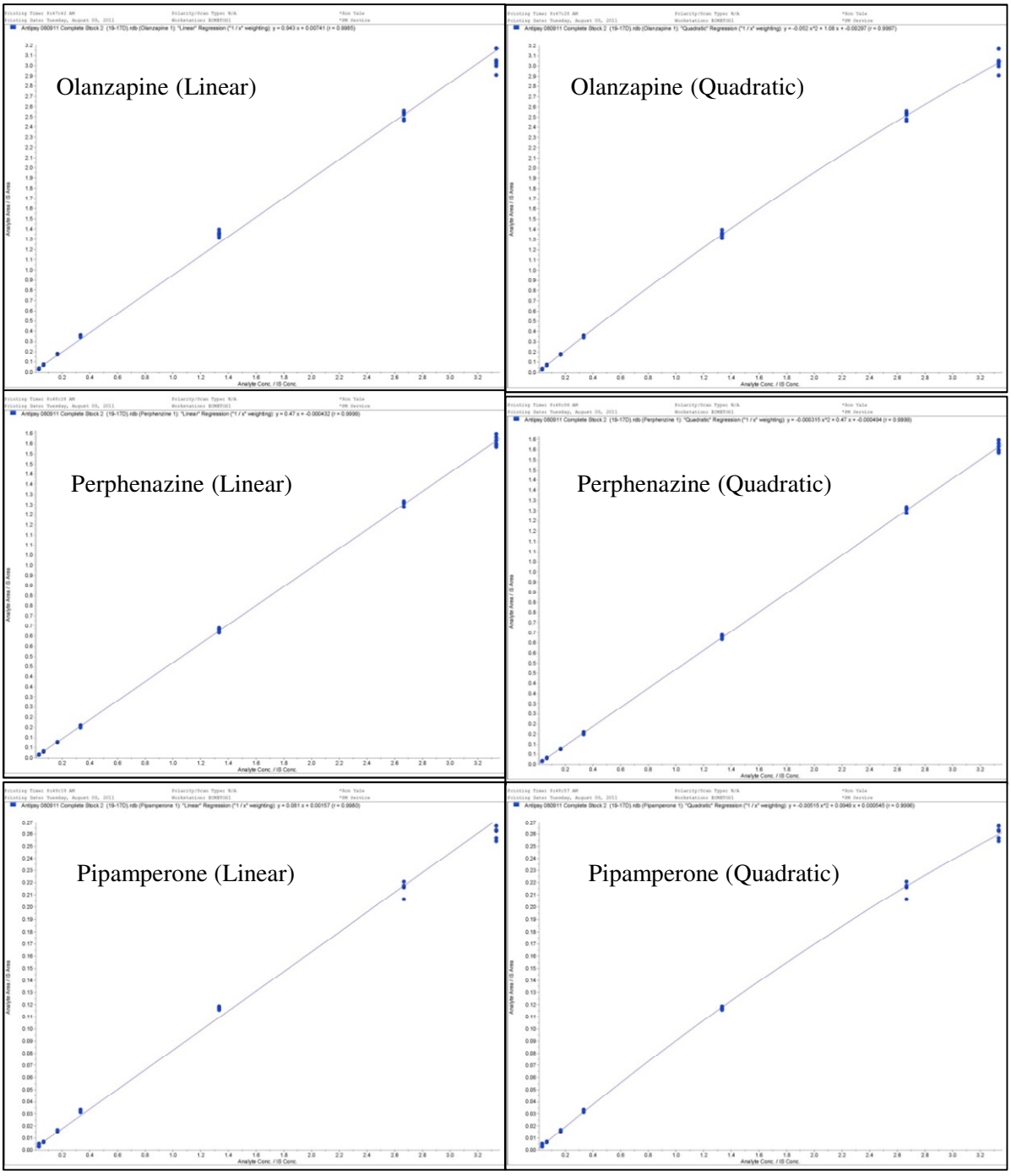
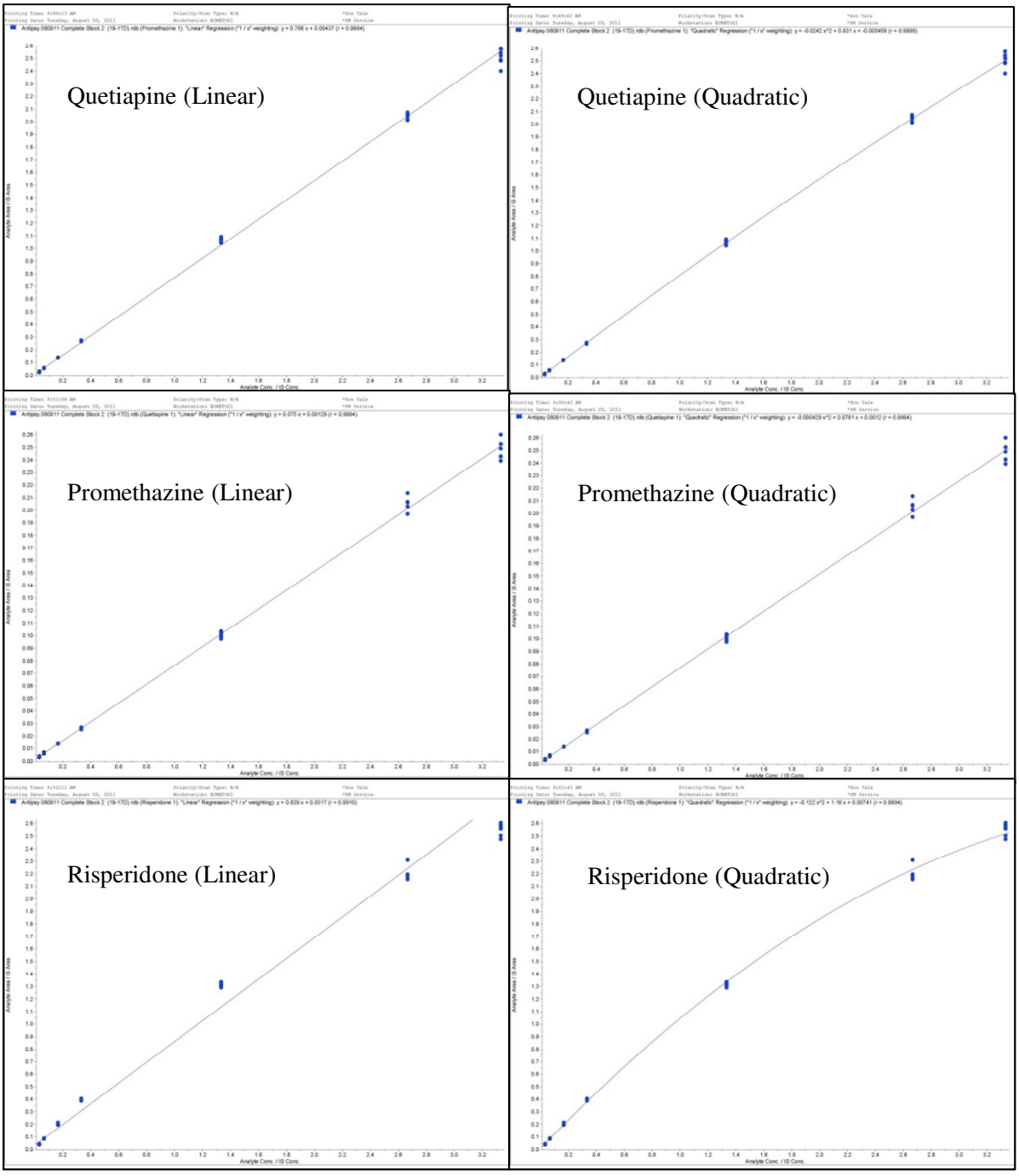
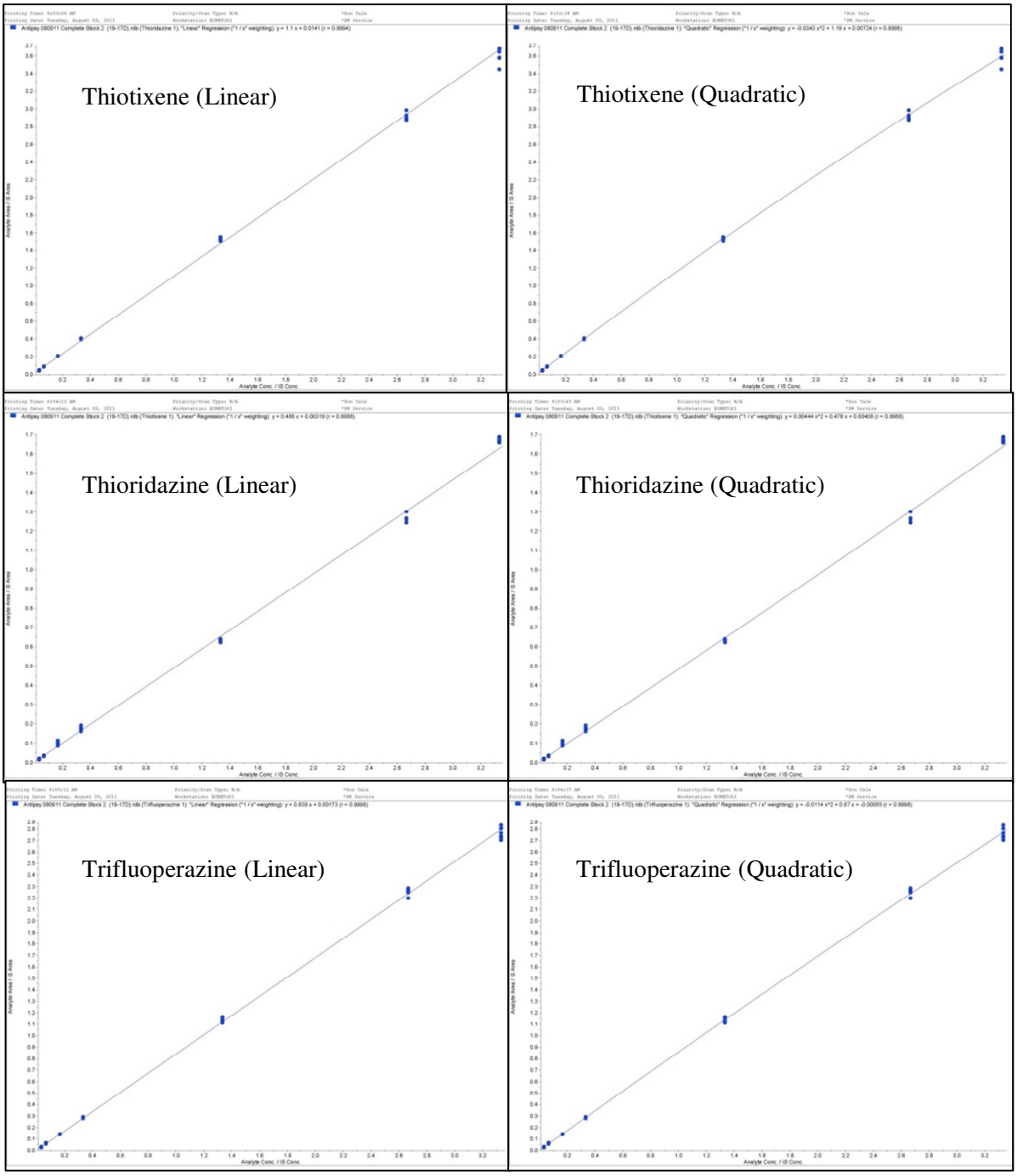
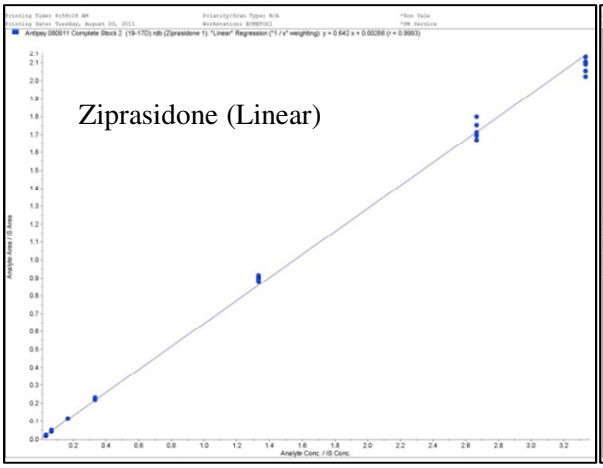
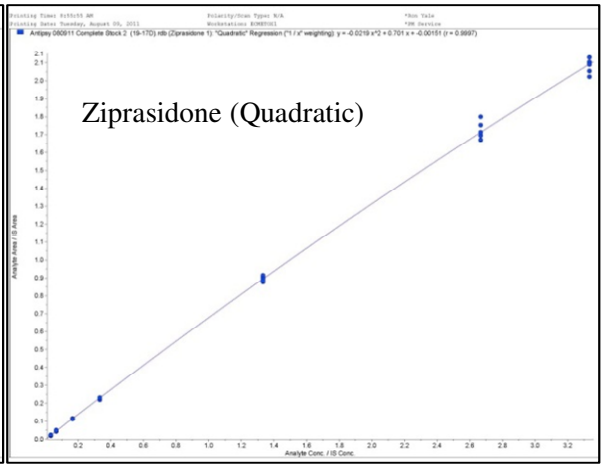
Haloperidol (Quadratic)
Loxapine (Linear)
Loxapine (Quadratic)
Mesoridazine (Linear)
Mesoridazine (Quadratic)


Olanzapine (Linear)
Olanzapine (Quadratic)
Perphenazine (Linear)
Perphenazine (Quadratic)
Pipamperone (Linear)
Pipamperone (Quadratic)


Quetiapine (Linear)
Quetiapine (Quadratic)
Promethazine (Linear)
Promethazine (Quadratic)
Risperidone (Linear)
Risperidone (Quadratic)


Thiotixene (Linear)
Thiotixene (Quadratic)
Thioridazine (Linear)
Thioridazine (Quadratic)
Trifluoperazine (Linear)
Trifluoperazine (Quadratic)


Ziprasidone (Linear)


Ziprasidone (Quadratic)

Graph Pad® Prism Software Calibration Model Data

	9-hydroxyrisperidone	Aripiprazole	Buspirone	Chlorpromazine
Comparison of Fits				
Null hypothesis	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
P value	< 0.0001	0.5759	< 0.0001	< 0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	Second order polynomial (quadratic)	First order polynomial (straight line)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
F (DFn, DFd)	608.7 (1,38)	0.3183 (1,38)	233.9 (1,38)	20.26 (1,38)
First order polynomial (straight line)				
Best-fit values				
B0	0.006731	0.0001091	0.0111	0.002083
B1	0.3036	0.0872	0.8175	1.007
Std. Error				
B0	0.001633	0.0001307	0.002836	0.00113
B1	0.004441	0.0003554	0.007712	0.003072
95% Confidence Intervals				
B0	0.003426 to 0.01003	-0.0001553 to 0.0003735	0.005360 to 0.01684	-0.0002027 to 0.004369
B1	0.2946 to 0.3126	0.08648 to 0.08791	0.8019 to 0.8331	1.001 to 1.013
Goodness of Fit				
Degrees of Freedom	39	39	39	39
R square (weighted)	0.9917	0.9994	0.9965	0.9996
Weighted Sum of Squares (1/X)	0.03067	0.0001964	0.09249	0.01468
Sy.x	0.02804	0.002244	0.0487	0.0194
Second order polynomial (quadratic)				
Best-fit values				
B0	0.000711	0.00006385	0.001104	-0.0004481
B1	0.3854	0.08781	0.9533	1.041
B2	-0.03014	-0.0002266	-0.05005	-0.01268
Std. Error				
B0	0.0004695	0.0001543	0.001257	0.001082
B1	0.003491	0.001147	0.009348	0.008045
B2	0.001222	0.0004017	0.003272	0.002816
95% Confidence Intervals				
B0	-0.0002398 to 0.001662	-0.0002487 to 0.0003764	-0.001442 to 0.003650	-0.002640 to 0.001743
B1	0.3783 to 0.3924	0.08549 to 0.09013	0.9344 to 0.9723	1.025 to 1.058
B2	-0.03262 to -0.02767	-0.001040 to 0.0005868	-0.05667 to -0.04342	-0.01838 to -0.006973
Goodness of Fit				
Degrees of Freedom	38	38	38	38
R square (weighted)	0.9995	0.9994	0.9995	0.9998
Weighted Sum of Squares (1/X)	0.001802	0.0001947	0.01292	0.009573
Sy.x	0.006886	0.002264	0.01844	0.01587
Number of points				
Analyzed	41	41	41	41

	Clozapine	Fluphenazine	Haloperidol	Loxapine
Comparison of Fits				
Null hypothesis	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
F (DFn, DFd)	223.2 (1,38)	19.18 (1,38)	136.9 (1,38)	258.1 (1,38)
First order polynomial (straight line)				
Best-fit values				
B0	0.01103	0.003397	0.01019	0.008873
B1	1.047	0.86	0.9534	0.9008
Std. Error				
B0	0.003047	0.001063	0.002605	0.002392
B1	0.008284	0.002892	0.007084	0.006505
95% Confidence Intervals				
B0	0.004867 to 0.01719	0.001245 to 0.005548	0.004915 to 0.01546	0.004033 to 0.01371
B1	1.030 to 1.064	0.8542 to 0.8658	0.9391 to 0.9678	0.8876 to 0.9140
Goodness of Fit				
Degrees of Freedom	39	39	39	39
R square (weighted)	0.9976	0.9996	0.9979	0.998
Weighted Sum of Squares (1/X)	0.1067	0.013	0.07804	0.0658
Sy.x	0.05231	0.01826	0.04473	0.04108
Second order polynomial (quadratic)				
Best-fit values				
B0	0.0003319	0.001057	0.00143	0.0003873
B1	1.192	0.8918	1.072	1.016
B2	-0.05357	-0.01172	-0.04385	-0.04249
Std. Error				
B0	0.001378	0.001028	0.00144	0.001016
B1	0.01025	0.007643	0.01071	0.007556
B2	0.003586	0.002675	0.003748	0.002645
95% Confidence Intervals				
B0	-0.002459 to 0.003123	-0.001025 to 0.003139	-0.001487 to 0.004346	-0.001671 to 0.002446
B1	1.171 to 1.213	0.8763 to 0.9073	1.051 to 1.094	1.001 to 1.031
B2	-0.06084 to -0.04631	-0.01714 to -0.006300	-0.05144 to -0.03626	-0.04785 to -0.03714
Goodness of Fit				
Degrees of Freedom	38	38	38	38
R square (weighted)	0.9996	0.9997	0.9995	0.9997
Weighted Sum of Squares (1/X)	0.01553	0.00864	0.01696	0.008444
Sy.x	0.02021	0.01508	0.02113	0.01491
Number of points				
Analyzed	41	41	41	41

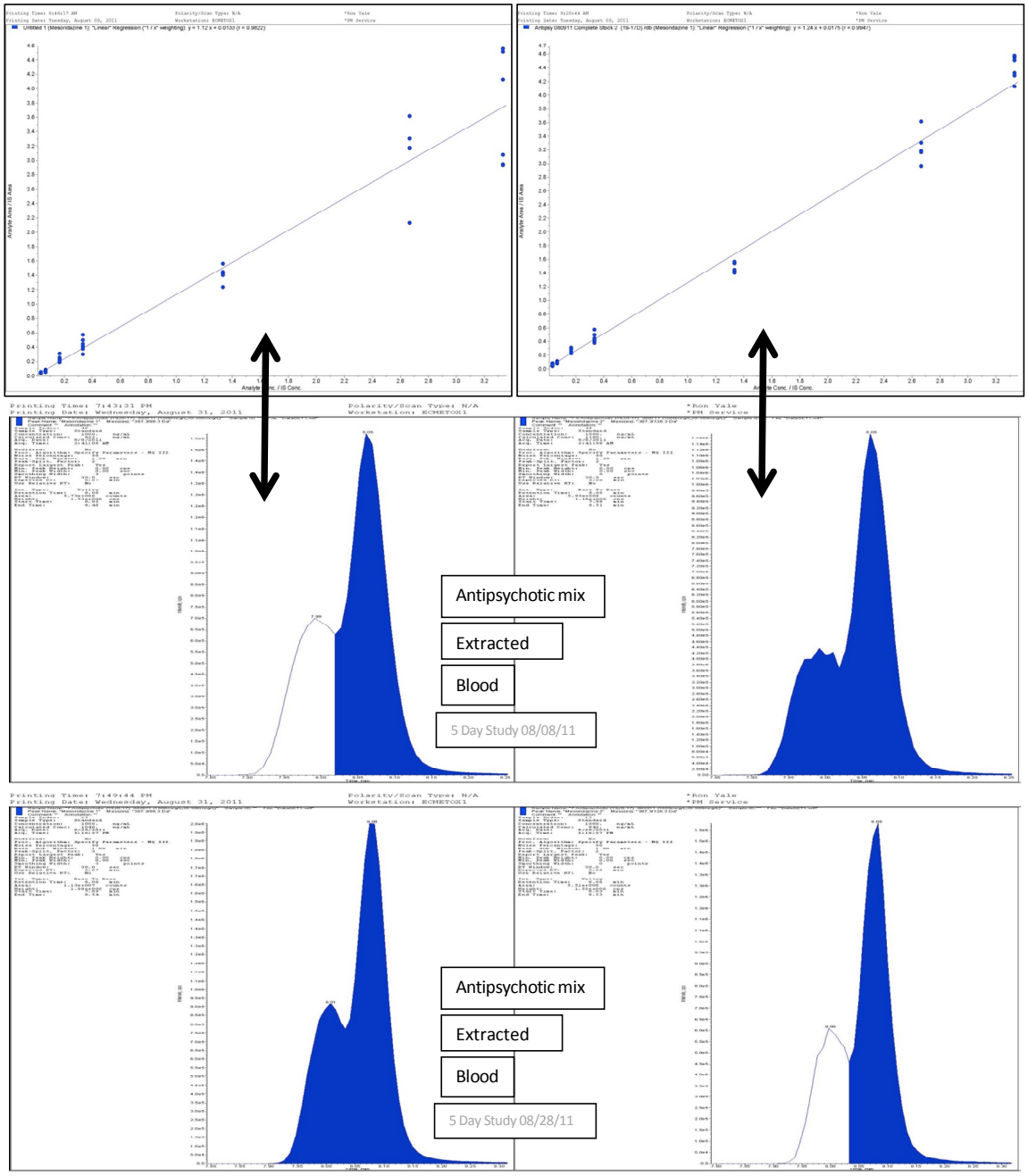
	Mesoridazine	Olanzapine	Perphenzine	Pipamperone
Comparison of Fits				
Null hypothesis	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
P value	0.0072	< 0.0001	0.9713	< 0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Second order polynomial (quadratic)	Second order polynomial (quadratic)	First order polynomial (straight line)	Second order polynomial (quadratic)
F (DFn, DFd)	8.074 (1,38)	170.8 (1,38)	0.001313 (1,38)	139.7 (1,38)
First order polynomial (straight line)				
Best-fit values				
B0	0.01759	0.007459	-0.0004807	0.001576
B1	1.244	0.9427	0.4698	0.08093
Std. Error				
B0	0.007532	0.003002	0.0004042	0.0003056
B1	0.02048	0.008161	0.001099	0.000831
95% Confidence Intervals				
B0	0.002352 to 0.03283	0.001386 to 0.01353	-0.001298 to 0.0003370	0.0009579 to 0.002195
B1	1.202 to 1.285	0.9261 to 0.9592	0.4676 to 0.4720	0.07925 to 0.08261
Goodness of Fit				
Degrees of Freedom	39	39	39	39
R square (weighted)	0.9895	0.9971	0.9998	0.9959
Weighted Sum of Squares (1/X)	0.6521	0.1036	0.001878	0.001074
Sy.x	0.1293	0.05153	0.00694	0.005248
Second order polynomial (quadratic)				
Best-fit values				
B0	0.02957	-0.002854	-0.0004717	0.0005467
B1	1.081	1.083	0.4697	0.09492
B2	0.05998	-0.05164	0.00004521	-0.005155
Std. Error				
B0	0.008111	0.001518	0.0004793	0.0001676
B1	0.0603	0.01129	0.003564	0.001246
B2	0.02111	0.003951	0.001247	0.0004361
95% Confidence Intervals				
B0	0.01314 to 0.04599	-0.005929 to 0.0002205	-0.001442 to 0.0004990	0.0002073 to 0.0008861
B1	0.9588 to 1.203	1.060 to 1.106	0.4625 to 0.4769	0.09240 to 0.09744
B2	0.01723 to 0.1027	-0.05965 to -0.04364	-0.002481 to 0.002571	-0.006039 to -0.004272
Goodness of Fit				
Degrees of Freedom	38	38	38	38
R square (weighted)	0.9914	0.9995	0.9998	0.9991
Weighted Sum of Squares (1/X)	0.5378	0.01885	0.001878	0.0002296
Sy.x	0.119	0.02227	0.00703	0.002458
Number of points				
Analyzed	41	41	41	41

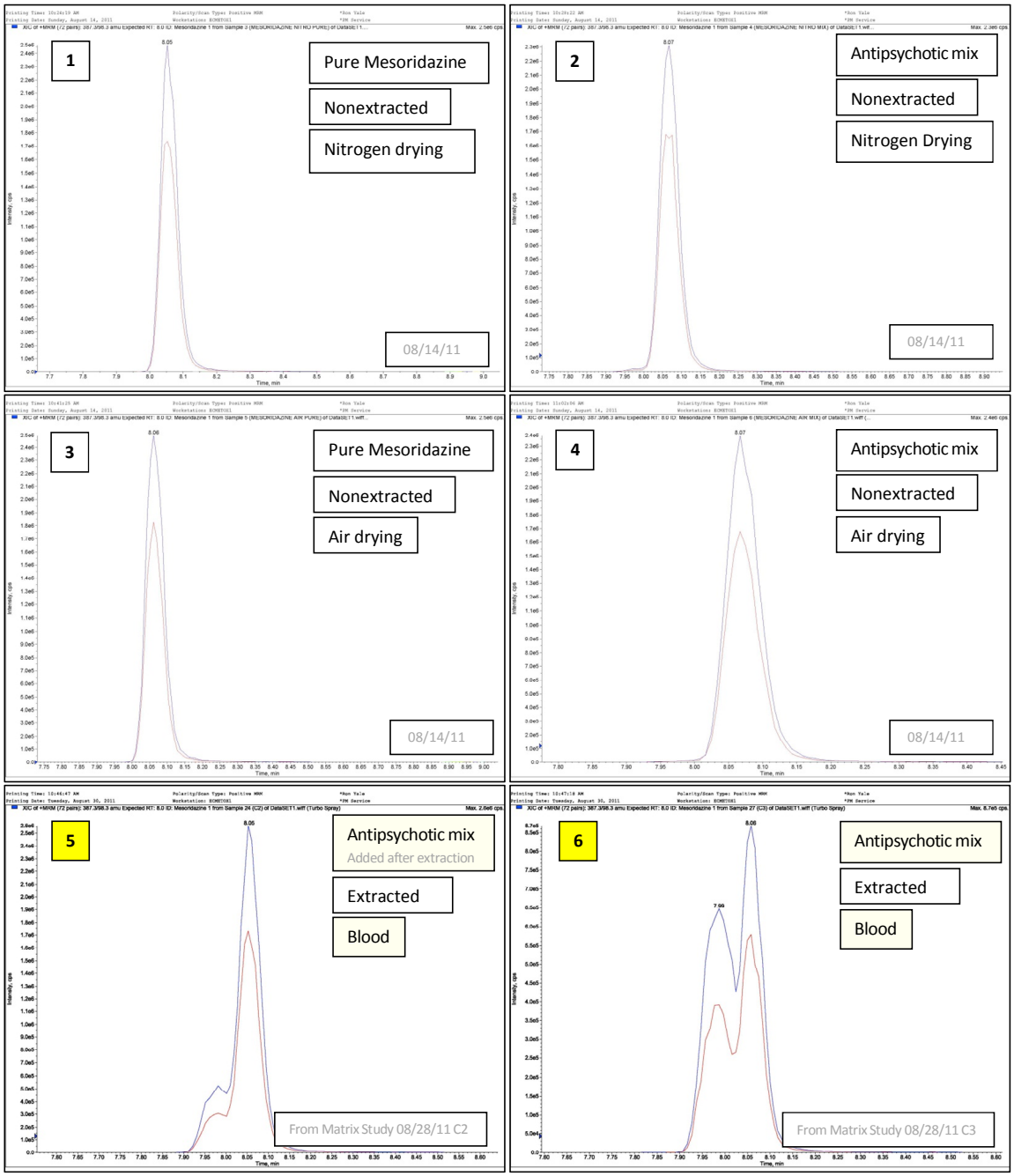
	Promethazine	Quetiapine	Risperidone	Thioridazine
Comparison of Fits				
Null hypothesis	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
P value	< 0.0001	0.3247	< 0.0001	< 0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	Second order polynomial (quadratic)	First order polynomial (straight line)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
F (DFn, DFd)	79.82 (1,38)	0.9956 (1,38)	599.9 (1,38)	79.62 (1,38)
First order polynomial (straight line)				
Best-fit values				
B0	0.004382	0.001284	0.03164	0.01412
B1	0.7655	0.07504	0.8295	1.097
Std. Error				
B0	0.001552	0.0001519	0.006624	0.002181
B1	0.00422	0.0004129	0.01801	0.005929
95% Confidence Intervals				
B0	0.001241 to 0.007522	0.0009773 to 0.001592	0.01824 to 0.04504	0.009710 to 0.01853
B1	0.7570 to 0.7740	0.07420 to 0.07587	0.7930 to 0.8659	1.085 to 1.109
Goodness of Fit				
Degrees of Freedom	39	39	39	39
R square (weighted)	0.9988	0.9988	0.9819	0.9989
Weighted Sum of Squares (1/X)	0.0277	0.0002651	0.5044	0.05466
Sy.x	0.02665	0.002607	0.1137	0.03744
Second order polynomial (quadratic)				
Best-fit values				
B0	-0.0004717	0.001192	0.007239	0.007306
B1	0.8315	0.07629	1.161	1.19
B2	-0.0243	-0.0004616	-0.1222	-0.03413
Std. Error				
B0	0.001045	0.0001778	0.001917	0.00147
B1	0.007772	0.001322	0.01425	0.01093
B2	0.00272	0.0004626	0.004989	0.003825
95% Confidence Intervals				
B0	-0.002589 to 0.001645	0.0008323 to 0.001552	0.003357 to 0.01112	0.004329 to 0.01028
B1	0.8157 to 0.8472	0.07361 to 0.07896	1.132 to 1.190	1.167 to 1.212
B2	-0.02981 to -0.01879	-0.001398 to 0.0004753	-0.1323 to -0.1121	-0.04187 to -0.02638
Goodness of Fit				
Degrees of Freedom	38	38	38	38
R square (weighted)	0.9996	0.9989	0.9989	0.9996
Weighted Sum of Squares (1/X)	0.008933	0.0002583	0.03004	0.01766
Sy.x	0.01533	0.002607	0.02812	0.02156
Number of points				
Analyzed	41	41	41	41

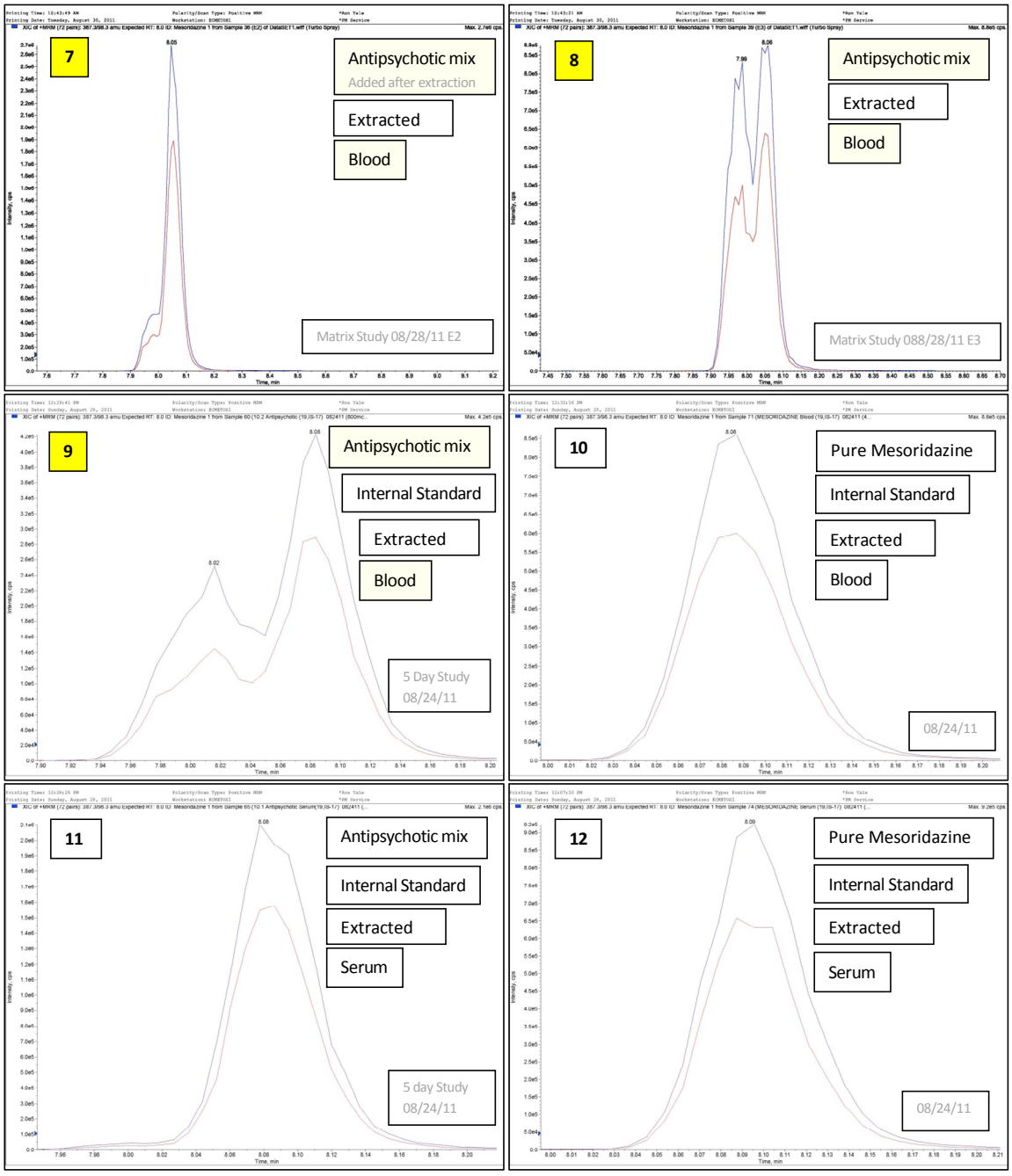
	Thiotixene	Trifluoperazine	Ziprasidone
Comparison of Fits			
Null hypothesis	First order polynomial (straight line)	First order polynomial (straight line)	First order polynomial (straight line)
Alternative hypothesis	Second order polynomial (quadratic)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
P value	0.2992	0.0003	< 0.0001
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	First order polynomial (straight line)	Second order polynomial (quadratic)	Second order polynomial (quadratic)
F (DFn, DFd)	1.108 (1,38)	15.54 (1,38)	67.64 (1,38)
First order polynomial (straight line)			
Best-fit values			
B0	0.00318	0.001747	0.002881
B1	0.4881	0.8387	0.6418
Std. Error			
B0	0.001416	0.001124	0.00143
B1	0.003851	0.003055	0.003887
95% Confidence Intervals			
B0	0.0003150 to 0.006045	-0.0005263 to 0.004020	-1.147e-005 to 0.005773
B1	0.4803 to 0.4959	0.8325 to 0.8449	0.6339 to 0.6496
Goodness of Fit			
Degrees of Freedom	39	39	39
R square (weighted)	0.9976	0.9995	0.9986
Weighted Sum of Squares (1/X)	0.02306	0.01451	0.02349
Sy.x	0.02431	0.01929	0.02454
Second order polynomial (quadratic)			
Best-fit values			
B0	0.004086	-0.0005529	-0.001465
B1	0.4758	0.87	0.7008
B2	0.004534	-0.01152	-0.02176
Std. Error			
B0	0.001655	0.001122	0.001017
B1	0.01231	0.008346	0.007559
B2	0.004308	0.002921	0.002646
95% Confidence Intervals			
B0	0.0007331 to 0.007438	-0.002826 to 0.001720	-0.003524 to 0.0005941
B1	0.4508 to 0.5007	0.8531 to 0.8869	0.6855 to 0.7161
B2	-0.004191 to 0.01326	-0.01743 to -0.005600	-0.02712 to -0.01640
Goodness of Fit			
Degrees of Freedom	38	38	38
R square (weighted)	0.9976	0.9996	0.9995
Weighted Sum of Squares (1/X)	0.0224	0.0103	0.008451
Sy.x	0.02428	0.01646	0.01491
Number of points			
Analyzed	41	41	41

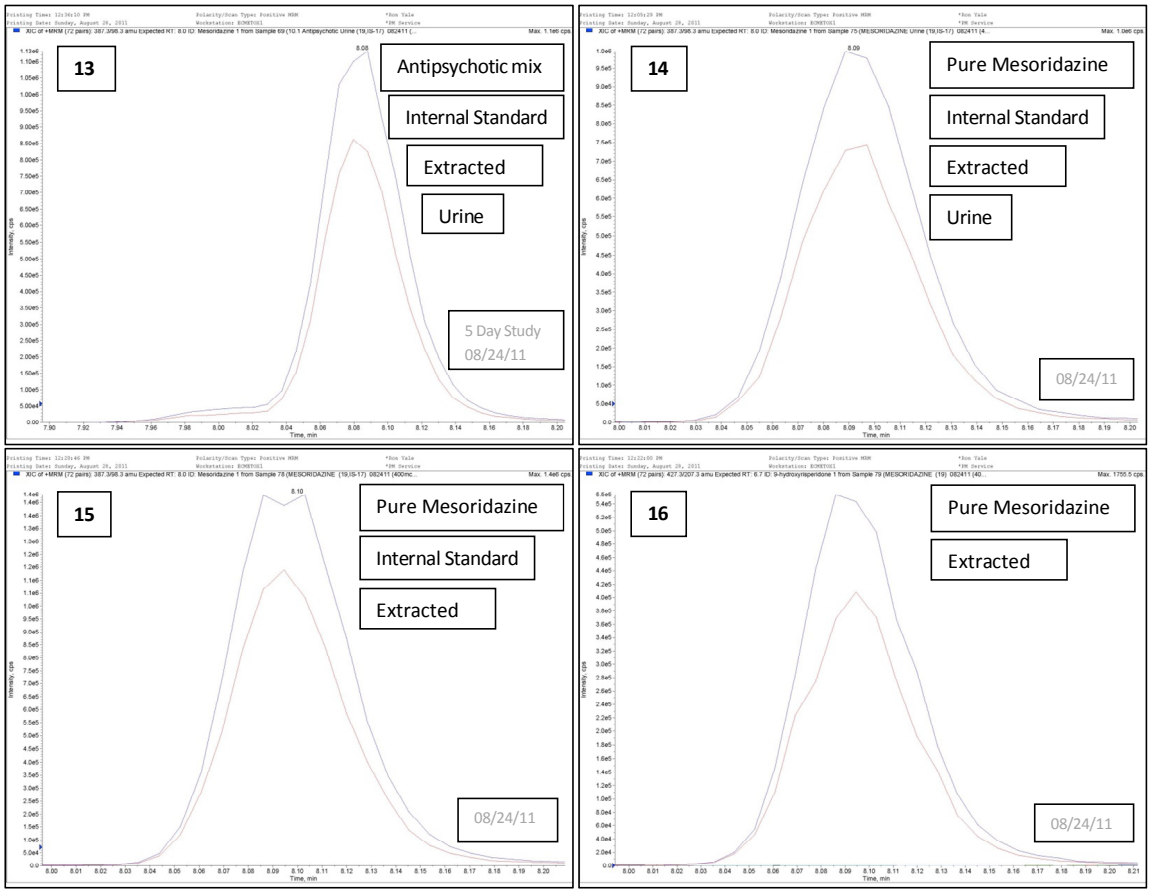
APPENDIX B

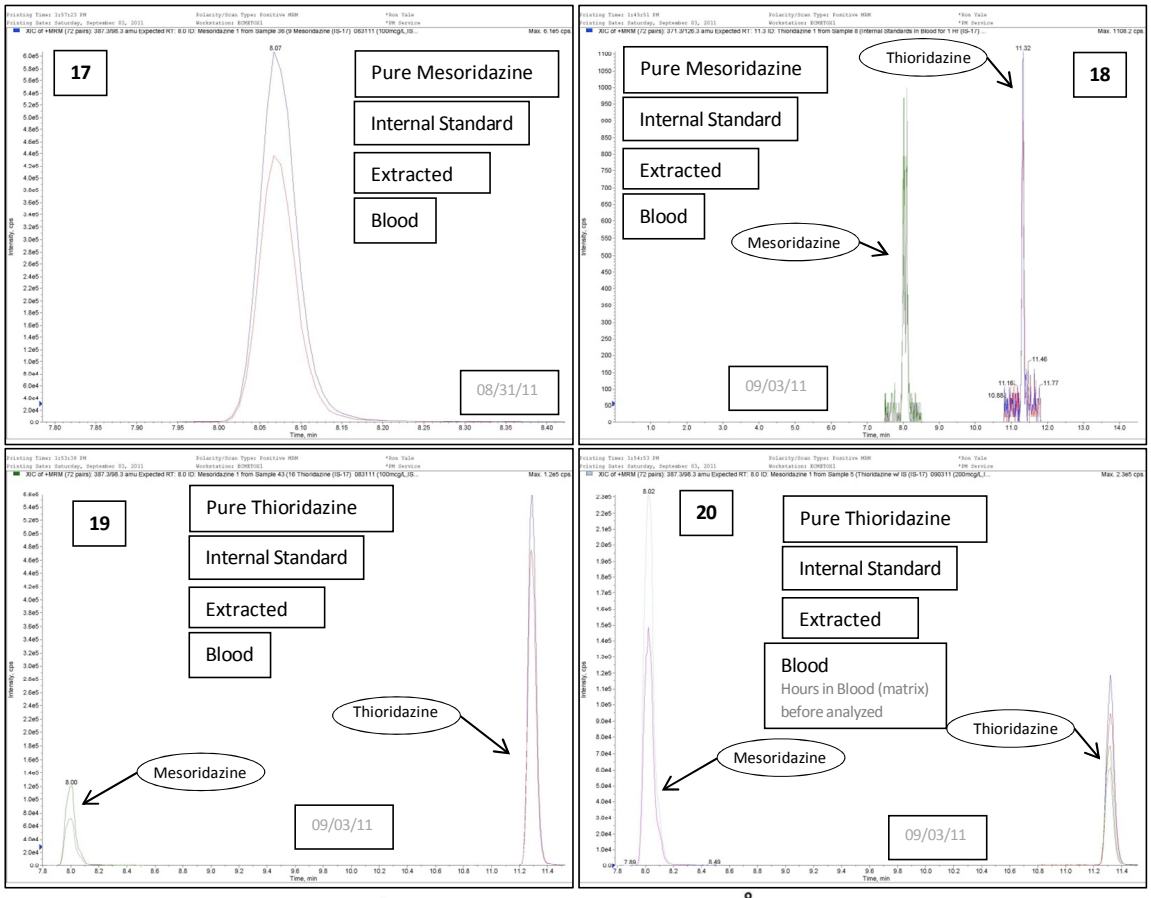
Mesoridazine Peak-Spitting study

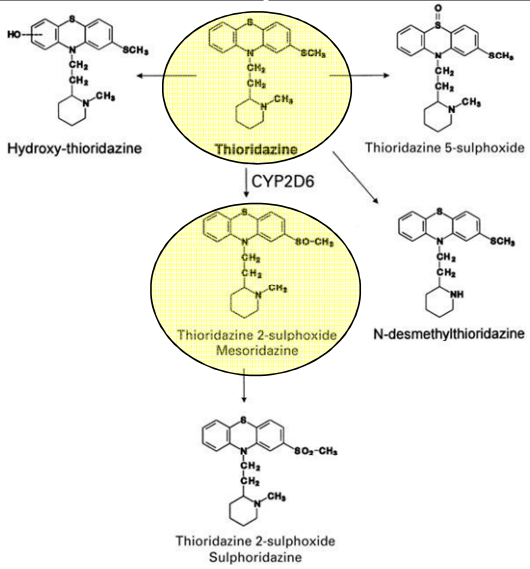



1
Pure Mesoridazine
Nonextracted
Nitrogen drying
08/14/11
2
Antipsychotic mix
Nonextracted
Nitrogen Drying
08/14/11
3
Pure Mesoridazine
Nonextracted
Air drying
08/14/11
4
Antipsychotic mix
Nonextracted
Air drying
08/14/11
5
Antipsychotic mix
Added after extraction
Extracted
Blood
From Matrix Study 08/28/11 C2
6
Antipsychotic mix
Extracted
Blood
From Matrix Study 08/28/11 C3

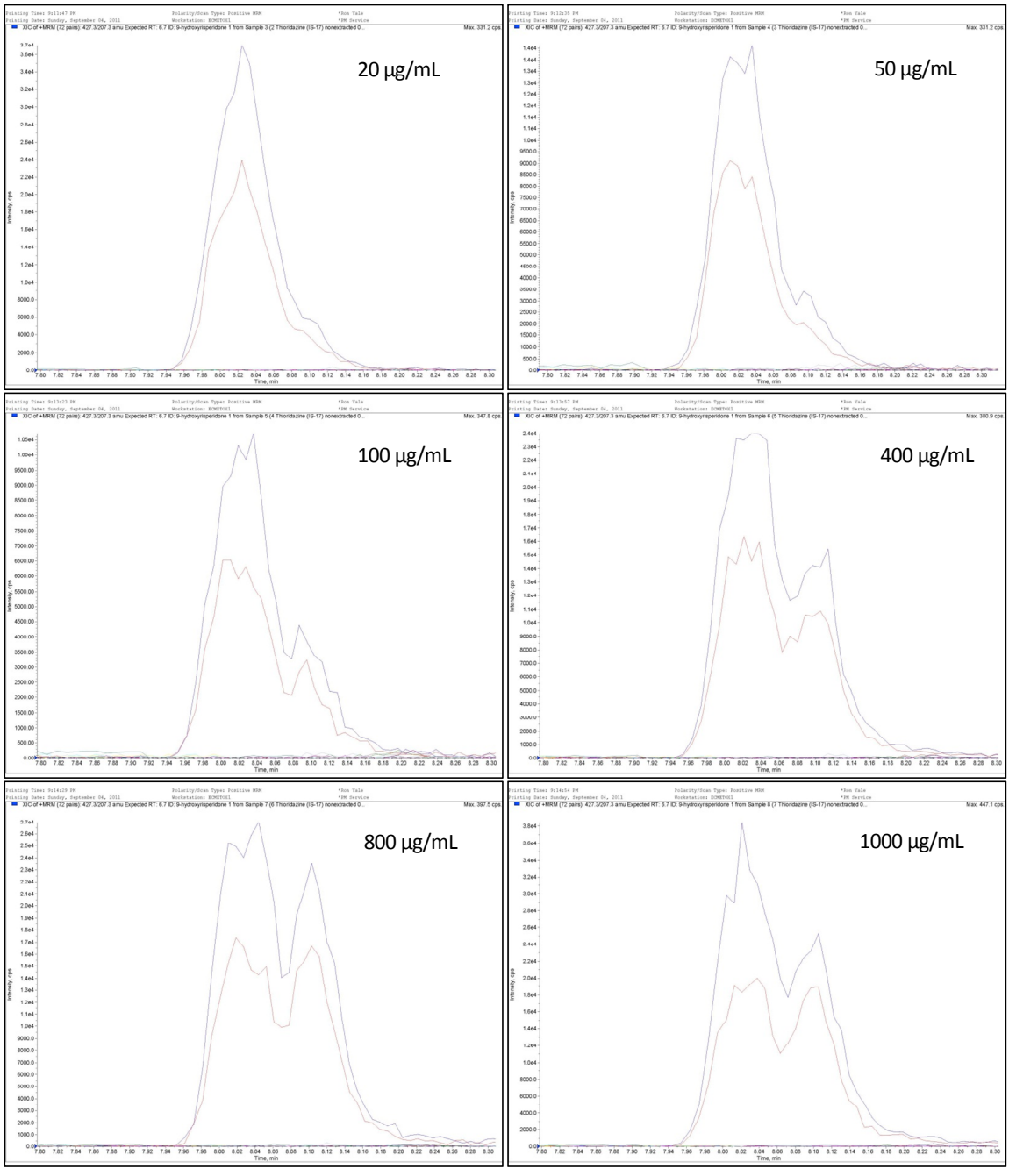

7
Antipsychotic mix
Added after extraction
Extracted
Blood
Matrix Study 08/28/11 E2
8
Antipsychotic mix
Extracted
Blood
Matrix Study 088/28/11 E3
9
Antipsychotic mix
Internal Standard
Extracted
Blood
5 Day Study
08/24/11
10
Pure Mesoridazine
Internal Standard
Extracted
Blood
08/24/11
11
Antipsychotic mix
Internal Standard
Extracted
Serum
5 day Study
08/24/11
12
Pure Mesoridazine
Internal Standard
Extracted
Serum
08/24/11


13
Antipsychotic mix
Internal Standard
Extracted
Urine
5 Day Study
08/24/11
14
Pure Mesoridazine
Internal Standard
Extracted
Urine
08/24/11
15
Pure Mesoridazine
Internal Standard
Extracted
08/24/11
16
Pure Mesoridazine
Extracted
08/24/11


17
Pure Mesoridazine
Internal Standard
Extracted
Blood
08/31/11
18
Pure Mesoridazine
Thioridazine
Internal Standard
Extracted
Blood
Mesoridazine
09/03/11
19
Pure Thioridazine
Internal Standard
Extracted
Blood
Thioridazine
Mesoridazine
09/03/11
20
Pure Thioridazine
Internal Standard
Extracted
Blood
Hours in Blood (matrix) before analyzed
Thioridazine
Mesoridazine
09/03/11


Hydroxy-thioridazine
Thioridazine
Thioridazine 5-sulphoxide
CYP2D6
Thioridazine 2-sulphoxide
Mesoridazine
N-desmethylthioridazine
Thioridazine 2-sulphoxide
Sulphoridazine

Quantified pure Thioridizine at different consentrations contained ~1% percent Mesoridizine.
Mesoridazine peaks of Pure nonextracted Thioridizine at concentrations of:



APPENDIX C

Within and Between Day Accuracy and Precision Data

9-hydroxyrisperidone																
Quadratic																
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.8	9.00%				21.2	6.00%				18.8	6.00%			
	20	21	5.00%	21.07	0.70	3.33%	21.6	8.00%	21.20	0.40	1.89%	19.5	2.50%	19.03	0.40	2.12%
	20	20.4	2.00%				20.8	4.00%				18.8	6.00%			
	100	101	1.00%				99.7	0.30%				103	3.00%			
	100	105	5.00%	104.33	3.06	2.93%	105	5.00%	100.43	4.25	4.23%	104	4.00%	101.43	3.61	3.56%
	100	107	7.00%				96.6	3.40%				97.3	2.70%			
	800	807	0.88%				782	2.25%				796	0.50%			
	800	824	3.00%	817.67	9.29	1.14%	755	5.63%	768.33	13.50	1.76%	801	0.13%	788.67	17.21	2.18%
	800	822	2.75%				768	4.00%				769	3.88%			
Serum	20	21.1	5.50%				20.5	2.50%				19.5	2.50%			
	100	101	1.00%				105	5.00%				103	3.00%			
	800	818	2.25%				781	2.38%				763	4.63%			
Urine	20	21.1	5.50%				22.1	10.50%				18.5	7.50%			
	100	101	1.00%				96.9	3.10%				105	5.00%			
	800	805	0.63%				771	3.63%				775	3.13%			
		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.1	0.50%				19.6	2.00%				20.5	2.50%			
	20	17.9	10.50%	19.00	1.10	5.79%	19.7	1.50%	19.73	0.15	0.77%	20.6	3.00%	20.03	0.90	4.47%
	20	19	5.00%				19.9	0.50%				19	5.00%			
	100	93.9	6.10%				104	4.00%				101	1.00%			
	100	95.4	4.60%	95.40	1.50	1.57%	97.5	2.50%	100.33	3.33	3.32%	95.2	4.80%	99.07	3.35	3.38%
	100	96.9	3.10%				99.5	0.50%				101	1.00%			
	800	781	2.38%				771	3.63%				798	0.25%			
	800	775	3.13%	768.67	16.44	2.14%	751	6.13%	773.67	24.11	3.12%	752	6.00%	775.33	23.01	2.97%
	800	750	6.25%				799	0.13%				776	3.00%			
Serum	20	19	5.00%				21.5	7.50%				20.5	2.50%			
	100	98.5	1.50%				107	7.00%				99	1.00%			
	800	754	5.75%				765	4.38%				804	0.50%			
Urine	20	19.2	4.00%				18.7	6.50%				18.5	7.50%			
	100	92.5	7.50%				111	11.00%				98.3	1.70%			
	800	740	7.50%				787	1.63%				765	4.38%			
		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day			
Blood	20	20.4	2.00%				21.1	5.50%								
	20	20.4	2.00%	20.37	0.06	0.28%			21.35	0.35	1.66%		20.17	1.02	5.06%	
	20	20.3	1.50%				21.6	8.00%								
	100	102	2.00%				107	7.00%								
	100	108	8.00%	106.33	3.79	3.56%	108	8.00%	106.67	1.53	1.43%		101.75	4.53	4.46%	
	100	109	9.00%				105	5.00%								
	800	800	0.00%				812	1.50%								
	800	819	2.38%	801.33	17.04	2.13%	802	0.25%	820.00	23.07	2.81%		789.21	25.65	3.25%	
	800	785	1.88%				846	5.75%								
Serum	20	19.9	0.50%				21.3	6.50%					20.41	0.89	4.36%	
	100	109	9.00%				112	12.00%					104.31	4.83	4.63%	
	800	809	1.13%				814	1.75%					788.50	25.72	3.26%	
Urine	20	19.5	2.50%				19.8	1.00%					19.68	1.30	6.63%	
	100	116	16.00%				110	10.00%					103.84	8.05	7.76%	
	800	797	0.38%				804	0.50%					780.50	22.26	2.85%	

Aripiprazole

Linear

		Day 1 (08/24/11) 19 mix					Day2 (08/28/11) 19 mix					Day3 (08/29/11) 19 mix				
	20	22.4	12.00%				22.4	12.00%				18.8	6.00%			
	20	21.4	7.00%	21.33	1.10	5.16%	21.1	5.50%	21.17	1.20	5.68%	18.5	7.50%	17.97	1.19	6.64%
	20	20.2	1.00%				20	0.00%				16.6	17.00%			
	100	98.5	1.50%				104	4.00%				100	0.00%			
Blood	100	104	4.00%	101.83	2.93	2.88%	106	6.00%	103.33	3.06	2.96%	99.6	0.40%	98.57	2.15	2.18%
	100	103	3.00%				100	0.00%				96.1	3.90%			
	800	820	2.50%				827	3.38%				801	0.13%			
	800	850	6.25%	835.33	15.01	1.80%	818	2.25%	821.33	4.93	0.60%	815	1.88%	812.00	9.85	1.21%
	800	836	4.50%				819	2.38%				820	2.50%			
	20	20.1	0.50%				21.1	5.50%				19.4	3.00%			
Serum	100	101	1.00%				103	3.00%				100	0.00%			
	800	840	5.00%				806	0.75%				807	0.88%			
	20	21	5.00%				22.1	10.50%				18.7	6.50%			
Urine	100	103	3.00%				104	4.00%				102	2.00%			
	800	784	2.00%				814	1.75%				813	1.63%			

		Day6 (09/22/11) 18 mix					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	23.3	16.50%				18.4	8.00%				21.3	6.50%			
	20	23.6	18.00%	22.63	1.42	6.28%	19.4	3.00%	18.43	0.95	5.16%	20.8	4.00%	21.33	0.55	2.58%
	20	21	5.00%				17.5	12.50%				21.9	9.50%			
	100	96.2	3.80%				94.8	5.20%				106	6.00%			
Blood	100	95	5.00%	95.57	0.60	0.63%	103	3.00%	99.93	4.47	4.48%	104	4.00%	105.33	1.15	1.10%
	100	95.5	4.50%				102	2.00%				106	6.00%			
	800	799	0.13%				807	0.88%				861	7.63%			
	800	782	2.25%	794.67	11.15	1.40%	786	1.75%	811.67	28.29	3.49%	846	5.75%	855.00	7.94	0.93%
	800	803	0.38%				842	5.25%				858	7.25%			
	20	21.5	7.50%				18.1	9.50%				20.2	1.00%			
Serum	100	101	1.00%				100	0.00%				110	10.00%			
	800	786	1.75%				785	1.88%				854	6.75%			
	20	25.4	27.00%				18	10.00%				20.9	4.50%			
Urine	100	105	5.00%				101	1.00%				106	6.00%			
	800	833	4.13%				810	1.25%				865	8.13%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	20.9	4.50%				20.5	2.50%						
	20			19.80	1.56	7.86%			20.70	0.28	1.37%	20.44	1.82	8.89%
	20	18.7	6.50%				20.9	4.50%						
	100	104	4.00%				106	6.00%						
Blood	100	99.4	0.60%	102.47	2.66	2.59%	99	1.00%	102.33	3.51	3.43%	101.17	3.75	3.70%
	100	104	4.00%				102	2.00%						
	800	788	1.50%				861	7.63%						
	800	791	1.13%	790.00	1.73	0.22%	842	5.25%	852.67	9.71	1.14%	821.58	25.89	3.15%
	800	791	1.13%				855	6.88%						
	20	19.2	4.00%				20	0.00%				19.95	1.08	5.39%
Serum	100	103	3.00%				105	5.00%				102.88	3.36	3.26%
	800	790	1.25%				832	4.00%				812.50	26.45	3.25%
	20	20.4	2.00%				19	5.00%				20.69	2.34	11.33%
Urine	100	106	6.00%				102	2.00%				103.63	1.92	1.86%
	800	783	2.13%				796	0.50%				812.25	27.10	3.34%

Buspirone																
		Quadratic														
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.1	5.50%	20.53	0.55	2.68%	20.7	3.50%	20.60	0.17	0.84%	19.1	4.50%	18.57	0.50	2.71%
	20	20	0.00%				20.4	2.00%				18.5	7.50%			
	20	20.5	2.50%				20.7	3.50%				18.1	9.50%			
	100	102	2.00%	103.00	2.65	2.57%	104	4.00%	103.00	1.00	0.97%	100	0.00%	101.33	1.53	1.51%
	100	101	1.00%				103	3.00%				103	3.00%			
	100	106	6.00%				102	2.00%				101	1.00%			
	800	779	2.63%	802.00	19.97	2.49%	789	1.38%	788.33	1.15	0.15%	810	1.25%	803.00	20.42	2.54%
	800	812	1.50%				789	1.38%				819	2.38%			
	800	815	1.88%				787	1.63%				780	2.50%			
Serum	20	20.8	4.00%				20.4	2.00%				18.5	7.50%			
	100	97.4	2.60%				106	6.00%				102	2.00%			
	800	752	6.00%				774	3.25%				765	4.38%			
Urine	20	20.5	2.50%				21.4	7.00%				17.4	13.00%			
	100	103	3.00%				102	2.00%				103	3.00%			
	800	758	5.25%				812	1.50%				791	1.13%			
		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.5	2.50%	19.63	0.90	4.59%	20.3	1.50%	20.50	0.26	1.29%	20	0.00%	20.23	0.32	1.59%
	20	19.7	1.50%				20.8	4.00%				20.6	3.00%			
	20	18.7	6.50%				20.4	2.00%				20.1	0.50%			
	100	97.8	2.20%	99.00	2.62	2.64%	104	4.00%	106.67	2.52	2.36%	98.3	1.70%	100.43	2.38	2.37%
	100	102	2.00%				109	9.00%				100	0.00%			
	100	97.2	2.80%				107	7.00%				103	3.00%			
	800	788	1.50%	788.33	1.53	0.19%	755	5.63%	780.33	33.26	4.26%	840	5.00%	831.33	21.36	2.57%
	800	787	1.63%				768	4.00%				807	0.88%			
	800	790	1.25%				818	2.25%				847	5.88%			
Serum	20	19.4	3.00%				21.2	6.00%				21.3	6.50%			
	100	98.2	1.80%				108	8.00%				101	1.00%			
	800	778	2.75%				815	1.88%				800	0.00%			
Urine	20	19	5.00%				19.6	2.00%				19.6	2.00%			
	100	97.5	2.50%				106	6.00%				105	5.00%			
	800	832	4.00%				764	4.50%				781	2.38%			
		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day			
Blood	20	19.7	1.50%	18.65	1.48	7.96%	20.4	2.00%	20.40	0.00	0.00%		19.92	0.94	4.72%	
	20															
	20	17.6	12.00%				20.4	2.00%								
	100	100	0.00%	101.00	1.73	1.71%	109	9.00%	107.00	1.73	1.62%		102.68	3.26	3.17%	
	100	100	0.00%				106	6.00%								
	100	103	3.00%				106	6.00%								
	800	777	2.88%	795.33	16.50	2.07%	835	4.38%	821.67	11.55	1.41%		801.29	22.81	2.85%	
	800	809	1.13%				815	1.88%								
	800	800	0.00%				815	1.88%								
Serum	20	19.7	1.50%				20.5	2.50%					20.23	0.96	4.76%	
	100	102	2.00%				105	5.00%					102.45	3.70	3.61%	
	800	821	2.63%				793	0.88%					787.25	24.21	3.08%	
Urine	20	18.5	7.50%				20.4	2.00%					19.55	1.26	6.44%	
	100	104	4.00%				106	6.00%					103.31	2.76	2.68%	
	800	776	3.00%				809	1.13%					790.38	25.60	3.24%	

Chlorpromazine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	21.5	7.50%				20.3	1.50%				19.1	4.50%			
	20	20.8	4.00%	20.73	0.80	3.87%	20.5	2.50%	20.27	0.25	1.24%	19	5.00%	18.67	0.67	3.57%
	20	19.9	0.50%				20	0.00%				17.9	10.50%			
	100	103	3.00%				101	1.00%				100	0.00%			
Blood	100	108	8.00%	105.33	2.52	2.39%	102	2.00%	101.00	1.00	0.99%	101	1.00%	100.33	0.58	0.58%
	100	105	5.00%				100	0.00%				100	0.00%			
	800	822	2.75%				799	0.13%				813	1.63%			
	800	799	0.13%	800.33	21.03	2.63%	827	3.38%	808.67	15.89	1.96%	818	2.25%	809.67	10.41	1.29%
	800	780	2.50%				800	0.00%				798	0.25%			
	20	20.7	3.50%				19.1	4.50%				19	5.00%			
Serum	100	102	2.00%				99.9	0.10%				98.6	1.40%			
	800	779	2.63%				790	1.25%				775	3.13%			
	20	21	5.00%				19.9	0.50%				17.5	12.50%			
Urine	100	106	6.00%				103	3.00%				99.4	0.60%			
	800	766	4.25%				804	0.50%				803	0.38%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20.6	3.00%				20.4	2.00%				20.3	1.50%			
	20	20.1	0.50%	20.03	0.60	3.01%	20.5	2.50%	20.20	0.44	2.16%	20.3	1.50%	20.17	0.23	1.15%
	20	19.4	3.00%				19.7	1.50%				19.9	0.50%			
	100	98.3	1.70%				102	2.00%				101	1.00%			
Blood	100	99.2	0.80%	98.73	0.45	0.46%	103	3.00%	102.33	0.58	0.56%	102	2.00%	102.33	1.53	1.49%
	100	98.7	1.30%				102	2.00%				104	4.00%			
	800	788	1.50%				766	4.25%				848	6.00%			
	800	793	0.88%	793.00	5.00	0.63%	790	1.25%	787.33	20.13	2.56%	855	6.88%	857.67	11.24	1.31%
	800	798	0.25%				806	0.75%				870	8.75%			
	20	20	0.00%				20.6	3.00%				21	5.00%			
Serum	100	98.9	1.10%				101	1.00%				103	3.00%			
	800	777	2.88%				785	1.88%				815	1.88%			
	20	20.5	2.50%				20.5	2.50%				19.7	1.50%			
Urine	100	103	3.00%				104	4.00%				104	4.00%			
	800	803	0.38%				773	3.38%				831	3.88%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	20	0.00%				20.9	4.50%						
	20			19.10	1.27	6.66%			20.75	0.21	1.02%	20.00	0.85	4.27%
	20	18.2	9.00%				20.6	3.00%						
	100	97.5	2.50%				98.2	1.80%						
Blood	100	96.7	3.30%	96.80	0.66	0.68%	99.1	0.90%	98.90	0.62	0.63%	100.72	2.72	2.71%
	100	96.2	3.80%				99.4	0.60%						
	800	793	0.88%				845	5.63%						
	800	797	0.38%	803.67	15.14	1.88%	796	0.50%	823.67	25.11	3.05%	810.50	25.22	3.11%
	800	821	2.63%				830	3.75%						
	20	19	5.00%				20.3	1.50%				19.96	0.82	4.12%
Serum	100	99.5	0.50%				103	3.00%				100.74	1.78	1.76%
	800	789	1.38%				804	0.50%				789.25	13.93	1.76%
	20	19.3	3.50%				20.9	4.50%				19.91	1.14	5.72%
Urine	100	103	3.00%				101	1.00%				102.93	2.00	1.94%
	800	763	4.63%				786	1.75%				791.13	23.34	2.95%

Clozapine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	21.9	9.50%				20.3	1.50%				18.8	6.00%			
	20	20.2	1.00%	20.83	0.93	4.46%	20.6	3.00%	20.13	0.57	2.82%	18.1	9.50%	18.27	0.47	2.59%
	20	20.4	2.00%				19.5	2.50%				17.9	10.50%			
	100	106	6.00%				102	2.00%				97.9	2.10%			
Blood	100	102	2.00%	104.67	2.31	2.21%	104	4.00%	102.33	1.53	1.49%	98.8	1.20%	97.50	1.54	1.58%
	100	106	6.00%				101	1.00%				95.8	4.20%			
	800	802	0.25%				789	1.38%				830	3.75%			
	800	819	2.38%	794.33	29.26	3.68%	815	1.88%	796.67	15.95	2.00%	805	0.63%	806.67	22.55	2.79%
	800	762	4.75%				786	1.75%				785	1.88%			
	20	20.5	2.50%				19.9	0.50%				18.8	6.00%			
Serum	100	98.5	1.50%				107	7.00%				96.7	3.30%			
	800	772	3.50%				808	1.00%				778	2.75%			
	20	20.4	2.00%				20.1	0.50%				16.8	16.00%			
Urine	100	94	6.00%				101	1.00%				96.8	3.20%			
	800	756	5.50%				785	1.88%				816	2.00%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20.1	0.50%				19.5	2.50%				20	0.00%			
	20	19.3	3.50%	19.63	0.42	2.12%	19.6	2.00%	19.53	0.06	0.30%	20.6	3.00%	20.17	0.38	1.88%
	20	19.5	2.50%				19.5	2.50%				19.9	0.50%			
	100	99.3	0.70%				101	1.00%				105	5.00%			
Blood	100	102	2.00%	99.73	2.08	2.09%	101	1.00%	102.67	2.89	2.81%	105	5.00%	105.67	1.15	1.09%
	100	97.9	2.10%				106	6.00%				107	7.00%			
	800	788	1.50%				767	4.13%				850	6.25%			
	800	778	2.75%	795.00	21.38	2.69%	777	2.88%	785.67	24.19	3.08%	814	1.75%	838.67	21.39	2.55%
	800	819	2.38%				813	1.63%				852	6.50%			
	20	19.4	3.00%				20.4	2.00%				21.2	6.00%			
Serum	100	100	0.00%				105	5.00%				104	4.00%			
	800	784	2.00%				812	1.50%				814	1.75%			
	20	17.9	10.50%				18.4	8.00%				19.3	3.50%			
Urine	100	96.5	3.50%				99	1.00%				108	8.00%			
	800	797	0.38%				769	3.88%				805	0.63%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	19.4	3.00%				20.7	3.50%						
	20	19.7	1.50%	19.20	0.62	3.25%			20.65	0.07	0.34%	19.77	0.91	4.58%
	20	18.5	7.50%				20.6	3.00%						
	100	99.5	0.50%				102	2.00%						
Blood	100	95.1	4.90%	97.53	2.24	2.29%	101	1.00%	102.00	1.00	0.98%	101.51	3.32	3.27%
	100	98	2.00%				103	3.00%						
	800	793	0.88%				862	7.75%						
	800	809	1.13%	794.00	14.53	1.83%	796	0.50%	836.33	35.36	4.23%	805.92	27.84	3.45%
	800	780	2.50%				851	6.38%						
	20	19.9	0.50%				20.3	1.50%				20.05	0.73	3.65%
Serum	100	97.7	2.30%				103	3.00%				101.49	3.77	3.72%
	800	782	2.25%				811	1.38%				795.13	17.66	2.22%
	20	19.6	2.00%				19.5	2.50%				19.00	1.21	6.38%
Urine	100	98.9	1.10%				98	2.00%				99.03	4.18	4.22%
	800	719	10.13%				766	4.25%				776.63	31.07	4.00%

Fluphenazine																
		Quadratic														
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.9	9.50%	21.80	0.26	1.21%	21.7	8.50%	20.90	0.70	3.35%	19.2	4.00%	18.47	0.81	4.38%
	20	22	10.00%				20.4	2.00%				18.6	7.00%			
	20	21.5	7.50%				20.6	3.00%				17.6	12.00%			
	100	100	0.00%	101.00	1.00	0.99%	106	6.00%	103.33	2.52	2.44%	98.9	1.10%	98.50	0.96	0.98%
	100	102	2.00%				103	3.00%				97.4	2.60%			
	100	101	1.00%				101	1.00%				99.2	0.80%			
	800	799	0.13%	820.33	29.50	3.60%	787	1.63%	797.33	20.55	2.58%	797	0.38%	806.00	15.59	1.93%
	800	854	6.75%				821	2.63%				797	0.38%			
	800	808	1.00%				784	2.00%				824	3.00%			
Serum	20	21.3	6.50%				20.1	0.50%				19.2	4.00%			
	100	102	2.00%				102	2.00%				99.6	0.40%			
	800	774	3.25%				783	2.13%				779	2.63%			
Urine	20	21.9	9.50%				21.7	8.50%				17.4	13.00%			
	100	104	4.00%				99	1.00%				98.8	1.20%			
	800	763	4.63%				788	1.50%				817	2.13%			
		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.8	4.00%	20.23	0.90	4.43%	21.2	6.00%	21.07	0.61	2.90%	20.4	2.00%	20.27	0.23	1.14%
	20	19.2	4.00%				20.4	2.00%				20.4	2.00%			
	20	20.7	3.50%				21.6	8.00%				20	0.00%			
	100	98.7	1.30%	100.13	1.69	1.69%	104	4.00%	106.33	2.52	2.37%	97	3.00%	100.67	3.21	3.19%
	100	102	2.00%				109	9.00%				102	2.00%			
	100	99.7	0.30%				106	6.00%				103	3.00%			
	800	801	0.13%	792.00	8.19	1.03%	756	5.50%	772.33	16.01	2.07%	810	1.25%	841.00	32.60	3.88%
	800	790	1.25%				773	3.38%				838	4.75%			
	800	785	1.88%				788	1.50%				875	9.38%			
Serum	20	20.9	4.50%				21.6	8.00%				21.3	6.50%			
	100	101	1.00%				110	10.00%				105	5.00%			
	800	773	3.38%				772	3.50%				779	2.63%			
Urine	20	20.8	4.00%				21.4	7.00%				20.7	3.50%			
	100	102	2.00%				104	4.00%				109	9.00%			
	800	804	0.50%				785	1.88%				780	2.50%			
		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day			
Blood	20	20.4	2.00%	19.37	0.96	4.96%	20.9	4.50%	20.95	0.07	0.34%		20.36	1.16	5.72%	
	20	19.2	4.00%													
	20	18.5	7.50%				21	5.00%								
	100	98.4	1.60%	96.93	3.17	3.27%	102	2.00%	100.63	1.58	1.57%		100.94	3.32	3.29%	
	100	93.3	6.70%				101	1.00%								
	100	99.1	0.90%				98.9	1.10%								
	800	776	3.00%	787.00	16.52	2.10%	851	6.38%	833.00	23.81	2.86%		806.13	28.97	3.59%	
	800	806	0.75%				806	0.75%								
	800	779	2.63%				842	5.25%								
Serum	20	19.6	2.00%				20.5	2.50%					20.56	0.87	4.22%	
	100	99.4	0.60%				104	4.00%					102.88	3.47	3.38%	
	800	771	3.63%				794	0.75%					778.13	7.64	0.98%	
Urine	20	20	0.00%				20.9	4.50%					20.60	1.43	6.93%	
	100	98.3	1.70%				104	4.00%					102.39	3.64	3.55%	
	800	744	7.00%				772	3.50%					781.63	22.87	2.93%	

Haloperidol

Quadratic

		Day1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	20.6	3.00%	20.80	0.72	3.47%	20.3	1.50%	20.00	0.44	2.18%	18.6	7.00%	18.10	0.87	4.78%
	20	20.2	1.00%				20.2	1.00%				18.6	7.00%			
	20	21.6	8.00%				19.5	2.50%				17.1	14.50%			
	100	96.5	3.50%	98.87	3.59	3.63%	105	5.00%	104.33	2.08	2.00%	103	3.00%	102.00	1.00	0.98%
	100	103	3.00%				106	6.00%				101	1.00%			
	100	97.1	2.90%				102	2.00%				102	2.00%			
	800	828	3.50%	825.67	11.68	1.41%	797	0.38%	787.00	12.49	1.59%	773	3.38%	774.67	2.08	0.27%
	800	813	1.63%				791	1.13%				777	2.88%			
	800	836	4.50%				773	3.38%				774	3.25%			
Serum	20	20.5	2.50%				20.9	4.50%				18.4	8.00%			
	100	95.1	4.90%				102	2.00%				100	0.00%			
	800	737	7.88%				800	0.00%				757	5.38%			
Urine	20	21	5.00%				21.5	7.50%				17.3	13.50%			
	100	97.9	2.10%				105	5.00%				103	3.00%			
	800	766	4.25%				807	0.88%				822	2.75%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	19.3	3.50%	19.07	0.40	2.12%	19.5	2.50%	19.87	0.40	2.03%	20.8	4.00%	20.80	0.20	0.96%
	20	19.3	3.50%				19.8	1.00%				21	5.00%			
	20	18.6	7.00%				20.3	1.50%				20.6	3.00%			
	100	100	0.00%	97.53	2.86	2.93%	104	4.00%	105.00	2.65	2.52%	110	10.00%	109.67	3.51	3.20%
	100	98.2	1.80%				108	8.00%				106	6.00%			
	100	94.4	5.60%				103	3.00%				113	13.00%			
	800	772	3.50%	776.67	4.51	0.58%	798	0.25%	801.00	11.79	1.47%	805	0.63%	820.67	15.50	1.89%
	800	777	2.88%				791	1.13%				821	2.63%			
	800	781	2.38%				814	1.75%				836	4.50%			
Serum	20	18.9	5.50%				20.2	1.00%				20.7	3.50%			
	100	95.2	4.80%				111	11.00%				107	7.00%			
	800	774	3.25%				794	0.75%				781	2.38%			
Urine	20	17.5	12.50%				19.5	2.50%				19.8	1.00%			
	100	96.1	3.90%				112	12.00%				112	12.00%			
	800	848	6.00%				808	1.00%				791	1.13%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day		
Blood	20	20.3	1.50%	19.85	0.64	3.21%	20.3	1.50%	19.95	0.49	2.48%		19.80	0.99	5.02%
	20														
	20	19.4	3.00%				19.6	2.00%							
	100	102	2.00%	103.67	2.08	2.01%	105	5.00%	103.33	1.53	1.48%		103.05	4.18	4.05%
	100	103	3.00%				102	2.00%							
	100	106	6.00%				103	3.00%							
	800	822	2.75%	818.67	3.06	0.37%	837	4.63%	829.00	38.63	4.66%		804.17	25.43	3.16%
	800	818	2.25%				787	1.63%							
	800	816	2.00%				863	7.88%							
Serum	20	19.9	0.50%				19.7	1.50%					19.90	0.88	4.41%
	100	103	3.00%				108	8.00%					102.66	5.82	5.67%
	800	820	2.50%				838	4.75%					787.63	32.75	4.16%
Urine	20	20.1	0.50%				20.4	2.00%					19.64	1.52	7.75%
	100	112	12.00%				106	6.00%					105.50	6.32	5.99%
	800	797	0.38%				855	6.88%					811.75	29.44	3.63%

Loxapine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	21	5.00%				19.5	2.50%				19.1	4.50%			
	20	20.5	2.50%	20.53	0.45	2.20%	19.7	1.50%	19.53	0.15	0.78%	18.3	8.50%	18.20	0.95	5.24%
	20	20.1	0.50%				19.4	3.00%				17.2	14.00%			
	100	97.5	2.50%				104	4.00%				103	3.00%			
Blood	100	101	1.00%	99.83	2.02	2.02%	101	1.00%	101.10	2.85	2.82%	103	3.00%	102.33	1.15	1.13%
	100	101	1.00%				98.3	1.70%				101	1.00%			
	800	837	4.63%				784	2.00%				796	0.50%			
	800	859	7.38%	836.67	22.50	2.69%	806	0.75%	797.33	11.72	1.47%	807	0.88%	797.67	8.62	1.08%
	800	814	1.75%				802	0.25%				790	1.25%			
	20	20.4	2.00%				19.2	4.00%				19.1	4.50%			
Serum	100	95.4	4.60%				102	2.00%				101	1.00%			
	800	774	3.25%				808	1.00%				767	4.13%			
	20	21.6	8.00%				20.1	0.50%				17.4	13.00%			
Urine	100	98.3	1.70%				100	0.00%				100	0.00%			
	800	747	6.63%				802	0.25%				817	2.13%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20.6	3.00%				19.5	2.50%				19.6	2.00%			
	20	19.6	2.00%	19.67	0.90	4.59%	20	0.00%	20.10	0.66	3.26%	19.8	1.00%	19.87	0.31	1.54%
	20	18.8	6.00%				20.8	4.00%				20.2	1.00%			
	100	98.8	1.20%				103	3.00%				104	4.00%			
Blood	100	100	0.00%	98.70	1.35	1.37%	106	6.00%	104.00	1.73	1.67%	99.5	0.50%	102.50	2.60	2.53%
	100	97.3	2.70%				103	3.00%				104	4.00%			
	800	783	2.13%				784	2.00%				824	3.00%			
	800	797	0.38%	795.67	12.06	1.52%	819	2.38%	810.00	22.87	2.82%	810	1.25%	832.33	27.47	3.30%
	800	807	0.88%				827	3.38%				863	7.88%			
	20	19.8	1.00%				20.5	2.50%				20.7	3.50%			
Serum	100	100	0.00%				108	8.00%				103	3.00%			
	800	774	3.25%				745	6.88%				803	0.38%			
	20	19.1	4.50%				19.8	1.00%				19.1	4.50%			
Urine	100	101	1.00%				103	3.00%				102	2.00%			
	800	817	2.13%				816	2.00%				807	0.88%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	19.5	2.50%				21.4	7.00%						
	20			19.30	0.28	1.47%			21.00	0.57	2.69%	19.74	0.94	4.75%
	20	19.1	4.50%				20.6	3.00%						
	100	101	1.00%				102	2.00%						
Blood	100	104	4.00%	102.00	1.73	1.70%	99.8	0.20%	100.93	1.10	1.09%	101.43	2.25	2.22%
	100	101	1.00%				101	1.00%						
	800	799	0.13%				871	8.88%						
	800	804	0.50%	794.67	12.10	1.52%	811	1.38%	842.00	30.05	3.57%	813.29	25.61	3.15%
	800	781	2.38%				844	5.50%						
	20	19.1	4.50%				19.9	0.50%				19.84	0.65	3.30%
Serum	100	102	2.00%				105	5.00%				102.05	3.68	3.60%
	800	777	2.88%				811	1.38%				782.38	23.00	2.94%
	20	19.4	3.00%				20.3	1.50%				19.60	1.20	6.15%
Urine	100	105	5.00%				102	2.00%				101.41	2.07	2.04%
	800	767	4.13%				833	4.13%				800.75	28.96	3.62%

Olanzapine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.4	7.00%	20.53	0.85	4.14%	20.4	2.00%	20.20	0.53	2.62%	18.4	8.00%	18.03	0.40	2.24%
	20	20.5	2.50%				20.6	3.00%				18.1	9.50%			
	20	19.7	1.50%				19.6	2.00%				17.6	12.00%			
	100	104	4.00%	104.00	0.00	0.00%	100	0.00%	99.33	2.08	2.10%	100	0.00%	102.00	2.00	1.96%
	100	104	4.00%				101	1.00%				104	4.00%			
	100	104	4.00%				97	3.00%				102	2.00%			
	800	785	1.88%	785.00	6.00	0.76%	808	1.00%	808.67	11.02	1.36%	818	2.25%	802.33	19.86	2.48%
	800	791	1.13%				820	2.50%				809	1.13%			
	800	779	2.63%				798	0.25%				780	2.50%			
Serum	20	21.6	8.00%				20.3	1.50%				18.1	9.50%			
	100	106	6.00%				103	3.00%				101	1.00%			
	800	718	10.25%				787	1.63%				761	4.88%			
Urine	20	20.8	4.00%				20.4	2.00%				18.1	9.50%			
	100	107	7.00%				99.7	0.30%				103	3.00%			
	800	721	9.88%				786	1.75%				794	0.75%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	17.3	13.50%	16.97	0.76	4.46%	20.1	0.50%	19.73	0.35	1.78%	19.8	1.00%	19.70	0.75	3.83%
	20	17.5	12.50%				19.7	1.50%				20.4	2.00%			
	20	16.1	19.50%				19.4	3.00%				18.9	5.50%			
	100	96.6	3.40%	95.03	1.38	1.45%	103	3.00%	104.33	1.53	1.46%	99.5	0.50%	100.83	1.26	1.25%
	100	94.5	5.50%				106	6.00%				101	1.00%			
	100	94	6.00%				104	4.00%				102	2.00%			
	800	790	1.25%	795.67	15.31	1.92%	772	3.50%	787.00	22.61	2.87%	804	0.50%	824.67	18.15	2.20%
	800	784	2.00%				776	3.00%				832	4.00%			
	800	813	1.63%				813	1.63%				838	4.75%			
Serum	20	16.4	18.00%				21	5.00%				21.3	6.50%			
	100	102	2.00%				103	3.00%				103	3.00%			
	800	775	3.13%				746	6.75%				786	1.75%			
Urine	20	18.3	8.50%				19.9	0.50%				20.3	1.50%			
	100	109	9.00%				106	6.00%				106	6.00%			
	800	829	3.63%				761	4.88%				780	2.50%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
Blood	20	18.6	7.00%	18.53	0.50	2.72%	21	5.00%	21.00	0.00	0.00%	19.27	1.38	7.18%
	20	19	5.00%											
	20	18	10.00%				21	5.00%						
	100	92.4	7.60%	92.93	2.44	2.63%	98.5	1.50%	100.50	1.80	1.79%	99.87	4.12	4.13%
	100	90.8	9.20%				102	2.00%						
	100	95.6	4.40%				101	1.00%						
	800	779	2.63%	798.33	17.79	2.23%	836	4.50%	814.00	25.53	3.14%	801.96	19.78	2.47%
	800	802	0.25%				786	1.75%						
	800	814	1.75%				820	2.50%						
Serum	20	18.8	6.00%				20.6	3.00%				19.76	1.82	9.22%
	100	96.7	3.30%				107	7.00%				102.71	3.14	3.06%
	800	773	3.38%				781	2.38%				765.88	23.68	3.09%
Urine	20	19.4	3.00%				22.3	11.50%				19.94	1.36	6.84%
	100	103	3.00%				106	6.00%				104.96	2.91	2.77%
	800	736	8.00%				774	3.25%				772.63	33.83	4.38%

Perphenzine

Linear

		Day 1 (08/24/11) 19 mix					Day2 (08/28/11) 19 mix					Day3 (08/29/11) 19 mix				
	20	21.3	6.50%				22	10.00%				18.6	7.00%			
	20	20.7	3.50%	20.87	0.38	1.81%	20.5	2.50%	20.73	1.17	5.63%	18.4	8.00%	18.13	0.64	3.55%
	20	20.6	3.00%				19.7	1.50%				17.4	13.00%			
	100	95.9	4.10%				107	7.00%				99.1	0.90%			
Blood	100	97.9	2.10%	96.60	1.13	1.17%	102	2.00%	104.67	2.52	2.40%	99.1	0.90%	99.40	0.52	0.52%
	100	96	4.00%				105	5.00%				100	0.00%			
	800	827	3.38%				823	2.88%				800	0.00%			
	800	847	5.88%	829.67	16.17	1.95%	836	4.50%	819.33	18.77	2.29%	802	0.25%	794.00	12.17	1.53%
	800	815	1.88%				799	0.13%				780	2.50%			
	20	21.3	6.50%				20	0.00%				19.2	4.00%			
Serum	100	95.1	4.90%				102	2.00%				97.8	2.20%			
	800	813	1.63%				798	0.25%				804	0.50%			
	20	22.8	14.00%				21.5	7.50%				17.6	12.00%			
Urine	100	102	2.00%				99.1	0.90%				102	2.00%			
	800	825	3.13%				821	2.63%				790	1.25%			

		Day6 (09/22/11) 18 mix					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20.8	4.00%				20	0.00%				20.7	3.50%			
	20	19.7	1.50%	20.03	0.67	3.32%	19.6	2.00%	19.90	0.26	1.33%	20.2	1.00%	20.30	0.36	1.78%
	20	19.6	2.00%				20.1	0.50%				20	0.00%			
	100	98.2	1.80%				101	1.00%				98	2.00%			
Blood	100	99.7	0.30%	98.20	1.50	1.53%	105	5.00%	105.33	4.51	4.28%	95.2	4.80%	98.07	2.90	2.96%
	100	96.7	3.30%				110	10.00%				101	1.00%			
	800	794	0.75%				755	5.63%				816	2.00%			
	800	803	0.38%	793.00	10.54	1.33%	790	1.25%	789.33	34.00	4.31%	852	6.50%	844.00	24.98	2.96%
	800	782	2.25%				823	2.88%				864	8.00%			
	20	21.1	5.50%				19.9	0.50%				21.4	7.00%			
Serum	100	104	4.00%				109	9.00%				107	7.00%			
	800	819	2.38%				789	1.38%				842	5.25%			
	20	20.2	1.00%				21.1	5.50%				20.9	4.50%			
Urine	100	99.8	0.20%				105	5.00%				108	8.00%			
	800	828	3.50%				821	2.63%				847	5.88%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	19.2	4.00%				20.2	1.00%						
	20			18.50	0.99	5.35%			20.25	0.07	0.35%	19.88	1.09	5.49%
	20	17.8	11.00%				20.3	1.50%						
	100	104	4.00%				103	3.00%						
Blood	100	100	0.00%	104.33	4.51	4.32%	98.1	1.90%	100.23	2.51	2.50%	100.85	4.05	4.02%
	100	109	9.00%				99.6	0.40%						
	800	798	0.25%				845	5.63%						
	800	813	1.63%	799.67	12.58	1.57%	805	0.63%	830.00	21.79	2.63%	812.38	26.18	3.22%
	800	788	1.50%				840	5.00%						
	20	19.7	1.50%				20.3	1.50%				20.36	0.81	4.00%
Serum	100	106	6.00%				107	7.00%				103.49	4.88	4.71%
	800	803	0.38%				817	2.13%				810.63	16.17	1.99%
	20	18.5	7.50%				20.7	3.50%				20.41	1.66	8.13%
Urine	100	112	12.00%				105	5.00%				104.11	4.34	4.16%
	800	777	2.88%				809	1.13%				814.75	22.26	2.73%

Pipamperone

Quadratic

		Day1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	20.6	3.00%	21.43	0.91	4.23%	22.4	12.00%	21.13	1.10	5.21%	18.4	8.00%	19.30	1.56	8.08%
	20	21.3	6.50%				20.6	3.00%				21.1	5.50%			
	20	22.4	12.00%				20.4	2.00%				18.4	8.00%			
	100	105	5.00%	104.33	1.15	1.11%	103	3.00%	103.67	1.15	1.11%	98	2.00%	100.13	2.58	2.58%
	100	105	5.00%				105	5.00%				99.4	0.60%			
	100	103	3.00%				103	3.00%				103	3.00%			
	800	824	3.00%	818.00	34.39	4.20%	802	0.25%	789.33	16.26	2.06%	797	0.38%	783.00	19.29	2.46%
	800	849	6.13%				795	0.63%				791	1.13%			
	800	781	2.38%				771	3.63%				761	4.88%			
Serum	20	20.9	4.50%				19.9	0.50%				19	5.00%			
	100	103	3.00%				98.9	1.10%				98.7	1.30%			
	800	732	8.50%				737	7.88%				742	7.25%			
Urine	20	21.1	5.50%				22.5	12.50%				17.5	12.50%			
	100	107	7.00%				104	4.00%				109	9.00%			
	800	741	7.38%				763	4.63%				795	0.63%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	19	5.00%	19.20	0.44	2.27%	20.4	2.00%	20.57	0.38	1.84%	19.1	4.50%	19.67	1.63	8.27%
	20	19.7	1.50%				20.3	1.50%				18.4	8.00%			
	20	18.9	5.50%				21	5.00%				21.5	7.50%			
	100	94	6.00%	94.97	1.12	1.18%	98.1	1.90%	98.37	3.51	3.57%	98.5	1.50%	99.00	1.80	1.82%
	100	94.7	5.30%				102	2.00%				97.5	2.50%			
	100	96.2	3.80%				95	5.00%				101	1.00%			
	800	788	1.50%	786.00	2.65	0.34%	759	5.13%	744.00	25.12	3.38%	795	0.63%	819.33	43.02	5.25%
	800	787	1.63%				715	10.63%				794	0.75%			
	800	783	2.13%				758	5.25%				869	8.63%			
Serum	20	20.7	3.50%				21.4	7.00%				20.6	3.00%			
	100	97.6	2.40%				101	1.00%				98.7	1.30%			
	800	755	5.63%				770	3.75%				761	4.88%			
Urine	20	20	0.00%				19.7	1.50%				19.5	2.50%			
	100	98.6	1.40%				100	0.00%				104	4.00%			
	800	833	4.13%				760	5.00%				740	7.50%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day		
Blood	20	20.6	3.00%	19.63	1.27	6.45%	21.3	6.50%	21.10	0.28	1.34%		20.22	1.25	6.20%
	20	20.1	0.50%												
	20	18.2	9.00%				20.9	4.50%							
	100	106	6.00%	101.53	5.35	5.27%	102	2.00%	101.33	2.08	2.05%		100.42	3.67	3.66%
	100	95.6	4.40%				99	1.00%							
	100	103	3.00%				103	3.00%							
	800	784	2.00%	788.67	50.16	6.36%	846	5.75%	823.00	47.84	5.81%		793.92	37.65	4.74%
	800	841	5.13%				768	4.00%							
	800	741	7.38%				855	6.88%							
Serum	20	18.1	9.50%				20.6	3.00%					20.15	1.10	5.44%
	100	98.5	1.50%				102	2.00%					99.80	1.94	1.94%
	800	792	1.00%				788	1.50%					759.63	22.58	2.97%
Urine	20	19.7	1.50%				19.8	1.00%					19.98	1.42	7.12%
	100	101	1.00%				105	5.00%					103.58	3.54	3.42%
	800	772	3.50%				752	6.00%					769.50	31.19	4.05%

Promethazine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	21.4	7.00%				20.6	3.00%				18.5	7.50%			
	20	21.2	6.00%	21.07	0.42	1.98%	20	0.00%	20.20	0.35	1.71%	18.6	7.00%	18.03	0.90	4.97%
	20	20.6	3.00%				20	0.00%				17	15.00%			
	100	102	2.00%				101	1.00%				103	3.00%			
Blood	100	102	2.00%	102.67	1.15	1.12%	101	1.00%	100.50	0.87	0.86%	103	3.00%	101.63	2.37	2.33%
	100	104	4.00%				99.5	0.50%				98.9	1.10%			
	800	807	0.88%				784	2.00%				805	0.63%			
	800	833	4.13%	812.00	19.00	2.34%	815	1.88%	797.00	16.09	2.02%	814	1.75%	802.67	12.66	1.58%
	800	796	0.50%				792	1.00%				789	1.38%			
	20	21.3	6.50%				19.5	2.50%				19	5.00%			
Serum	100	104	4.00%				99.4	0.60%				98	2.00%			
	800	768	4.00%				782	2.25%				767	4.13%			
	20	21.8	9.00%				21.1	5.50%				17.6	12.00%			
Urine	100	105	5.00%				101	1.00%				102	2.00%			
	800	771	3.63%				790	1.25%				797	0.38%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20.3	1.50%				20.4	2.00%				20	0.00%			
	20	19.2	4.00%	19.73	0.55	2.79%	19.8	1.00%	20.20	0.35	1.71%	21.2	6.00%	20.33	0.76	3.72%
	20	19.7	1.50%				20.4	2.00%				19.8	1.00%			
	100	94.9	5.10%				101	1.00%				99.9	0.10%			
Blood	100	100	0.00%	97.57	2.56	2.62%	103	3.00%	102.00	1.00	0.98%	98.1	1.90%	100.33	2.48	2.47%
	100	97.8	2.20%				102	2.00%				103	3.00%			
	800	791	1.13%				740	7.50%				834	4.25%			
	800	784	2.00%	788.00	3.61	0.46%	756	5.50%	763.67	28.29	3.70%	851	6.38%	856.67	25.97	3.03%
	800	789	1.38%				795	0.63%				885	10.63%			
	20	19.6	2.00%				20.7	3.50%				20.4	2.00%			
Serum	100	103	3.00%				105	5.00%				102	2.00%			
	800	780	2.50%				760	5.00%				843	5.38%			
	20	20	0.00%				20.3	1.50%				19	5.00%			
Urine	100	101	1.00%				104	4.00%				106	6.00%			
	800	793	0.88%				786	1.75%				815	1.88%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	19.6	2.00%				20.6	3.00%						
	20			19.20	0.57	2.95%			20.30	0.42	2.09%	19.90	1.02	5.11%
	20	18.8	6.00%				20	0.00%						
	100	99.7	0.30%				106	6.00%						
Blood	100	99.8	0.20%	100.17	0.72	0.72%	103	3.00%	104.00	1.73	1.67%	101.11	2.36	2.34%
	100	101	1.00%				103	3.00%						
	800	791	1.13%				849	6.13%						
	800	805	0.63%	805.33	14.50	1.80%	804	0.50%	829.67	23.16	2.79%	806.88	31.02	3.84%
	800	820	2.50%				836	4.50%						
	20	19.7	1.50%				20.8	4.00%				20.13	0.79	3.92%
Serum	100	101	1.00%				108	8.00%				102.55	3.19	3.11%
	800	801	0.13%				820	2.50%				790.13	29.05	3.68%
	20	20	0.00%				21.3	6.50%				20.14	1.35	6.73%
Urine	100	106	6.00%				106	6.00%				103.88	2.23	2.15%
	800	777	2.88%				798	0.25%				790.88	13.56	1.71%

Quetiapine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	20.4	2.00%	20.87	0.40	1.94%	18.9	5.50%	19.70	0.85	4.34%	19.3	3.50%	19.20	2.05	10.69%
	20	21.1	5.50%				20.6	3.00%				21.2	6.00%			
	20	21.1	5.50%				19.6	2.00%				17.1	14.50%			
	100	101	1.00%	101.33	0.58	0.57%	97.8	2.20%	97.93	3.00	3.07%	97.9	2.10%	101.63	3.27	3.22%
	100	102	2.00%				101	1.00%				104	4.00%			
	100	101	1.00%				95	5.00%				103	3.00%			
	800	843	5.38%	847.33	5.13	0.61%	817	2.13%	810.33	9.07	1.12%	775	3.13%	788.00	11.27	1.43%
	800	853	6.63%				814	1.75%				794	0.75%			
	800	846	5.75%				800	0.00%				795	0.63%			
Serum	20	20.3	1.50%				19.3	3.50%				20.1	0.50%			
	100	97.1	2.90%				98.1	1.90%				93.9	6.10%			
	800	812	1.50%				753	5.88%				788	1.50%			
Urine	20	21.6	8.00%				20.2	1.00%				15.1	24.50%			
	100	99.3	0.70%				92.8	7.20%				99.8	0.20%			
	800	805	0.63%				779	2.63%				783	2.13%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.8	4.00%	19.87	0.81	4.10%	19.2	4.00%	19.80	1.31	6.60%	20.2	1.00%	20.50	0.30	1.46%
	20	19.5	2.50%				18.9	5.50%				20.8	4.00%			
	20	19.3	3.50%				21.3	6.50%				20.5	2.50%			
	100	96	4.00%	98.67	2.31	2.34%	96.7	3.30%	100.23	3.22	3.21%	105	5.00%	106.00	4.58	4.32%
	100	100	0.00%				101	1.00%				102	2.00%			
	100	100	0.00%				103	3.00%				111	11.00%			
	800	797	0.38%	790.33	11.55	1.46%	805	0.63%	830.67	23.46	2.82%	799	0.13%	842.00	46.89	5.57%
	800	777	2.88%				851	6.38%				835	4.38%			
	800	797	0.38%				836	4.50%				892	11.50%			
Serum	20	19.5	2.50%				18.8	6.00%				21.3	6.50%			
	100	101	1.00%				101	1.00%				105	5.00%			
	800	803	0.38%				813	1.63%				774	3.25%			
Urine	20	19.6	2.00%				16.1	19.50%				17.5	12.50%			
	100	99.4	0.60%				80.3	19.70%				93.3	6.70%			
	800	805	0.63%				664	17.00%				687	14.13%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day			
Blood	20	19.3	3.50%	18.35	1.34	7.32%	23.4	17.00%	22.35	1.48	6.64%	20.05	1.40	6.96%	
	20														
	20	17.4	13.00%				21.3	6.50%							
	100	104	4.00%	103.67	0.58	0.56%	106	6.00%	113.67	7.51	6.60%	102.89	5.77	5.61%	
	100	103	3.00%				114	14.00%							
	100	104	4.00%				121	21.00%							
	800	758	5.25%	764.67	7.02	0.92%	826	3.25%	872.00	41.90	4.81%	818.17	40.16	4.91%	
	800	772	3.50%				882	10.25%							
	800	764	4.50%				908	13.50%							
Serum	20	19.7	1.50%				20.3	1.50%				19.91	0.76	3.84%	
	100	101	1.00%				97.4	2.60%				99.31	3.39	3.41%	
	800	764	4.50%				827	3.38%				791.75	26.27	3.32%	
Urine	20	14.6	27.00%				15.3	23.50%				17.50	2.66	15.19%	
	100	78.6	21.40%				73.8	26.20%				89.66	10.52	11.74%	
	800	577	27.88%				613	23.38%				714.13	90.83	12.72%	

Risperidone

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	20.8	**4.00%**				20.5	**2.50%**				18.8	**6.00%**			
	20	21.4	**7.00%**	21.13	0.31	**1.45%**	20.7	**3.50%**	20.47	0.25	**1.23%**	18.6	**7.00%**	18.33	0.64	**3.51%**
	20	21.2	**6.00%**				20.2	**1.00%**				17.6	**12.00%**			
	100	104	**4.00%**				105	**5.00%**				105	**5.00%**			
Blood	**100**	105	**5.00%**	104.67	0.58	**0.55%**	107	**7.00%**	105.00	2.00	**1.90%**	107	**7.00%**	105.33	1.53	**1.45%**
	100	105	**5.00%**				103	**3.00%**				104	**4.00%**			
	800	788	**1.50%**				773	**3.38%**				790	**1.25%**			
	800	851	**6.38%**	802.00	43.71	**5.45%**	794	**0.75%**	777.00	15.39	**1.98%**	763	**4.63%**	778.33	13.87	**1.78%**
	800	767	**4.13%**				764	**4.50%**				782	**2.25%**			
	20	21	**5.00%**				20.7	**3.50%**				18.3	**8.50%**			
Serum	**100**	105	**5.00%**				105	**5.00%**				105	**5.00%**			
	800	715	**10.63%**				804	**0.50%**				744	**7.00%**			
	20	21.1	**5.50%**				21.5	**7.50%**				17.4	**13.00%**			
Urine	**100**	102	**2.00%**				103	**3.00%**				105	**5.00%**			
	800	711	**11.13%**				761	**4.88%**				813	**1.63%**			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	20	**0.00%**				19.6	**2.00%**				21.1	**5.50%**			
	20	18.9	**5.50%**	19.13	0.78	**4.06%**	19.8	**1.00%**	19.83	0.25	**1.27%**	21.2	**6.00%**	20.57	1.01	**4.92%**
	20	18.5	**7.50%**				20.1	**0.50%**				19.4	**3.00%**			
	100	98.6	**1.40%**				105	**5.00%**				105	**5.00%**			
Blood	**100**	101	**1.00%**	99.80	1.20	**1.20%**	107	**7.00%**	107.33	2.52	**2.34%**	103	**3.00%**	105.33	2.52	**2.39%**
	100	99.8	**0.20%**				110	**10.00%**				108	**8.00%**			
	800	752	**6.00%**				699	**12.63%**				731	**8.63%**			
	800	751	**6.13%**	759.00	13.00	**1.71%**	677	**15.38%**	703.00	28.21	**4.01%**	791	**1.13%**	804.00	80.29	**9.99%**
	800	774	**3.25%**				733	**8.38%**				890	**11.25%**			
	20	18.8	**6.00%**				20.2	**1.00%**				21.7	**8.50%**			
Serum	**100**	99	**1.00%**				108	**8.00%**				104	**4.00%**			
	800	734	**8.25%**				711	**11.13%**				730	**8.75%**			
	20	17.9	**10.50%**				19.3	**3.50%**				18.9	**5.50%**			
Urine	**100**	98.4	**1.60%**				110	**10.00%**				105	**5.00%**			
	800	782	**2.25%**				709	**11.38%**				706	**11.75%**			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix					Between Day		
	20	20	**0.00%**				20.9	**4.50%**						
	20			19.25	1.06	**5.51%**			20.80	0.14	**0.68%**	19.93	1.07	**5.38%**
	20	18.5	**7.50%**				20.7	**3.50%**						
	100	102	**2.00%**				106	**6.00%**						
Blood	**100**	104	**4.00%**	104.00	2.00	**1.92%**	111	**11.00%**	108.00	2.65	**2.45%**	104.93	2.89	**2.75%**
	100	106	**6.00%**				107	**7.00%**						
	800	727	**9.13%**				876	**9.50%**						
	800	787	**1.63%**	753.00	30.79	**4.09%**	757	**5.38%**	817.33	59.52	**7.28%**	774.21	49.51	**6.39%**
	800	745	**6.88%**				819	**2.38%**						
	20	19.4	**3.00%**				20.2	**1.00%**				20.04	1.14	**5.70%**
Serum	**100**	104	**4.00%**				109	**9.00%**				104.88	3.00	**2.86%**
	800	762	**4.75%**				742	**7.25%**				742.75	29.62	**3.99%**
	20	19.1	**4.50%**				20.3	**1.50%**				19.44	1.45	**7.46%**
Urine	**100**	110	**10.00%**				107	**7.00%**				105.05	3.97	**3.78%**
	800	681	**14.88%**				755	**5.63%**				739.75	45.04	**6.09%**

Thioridazine																
		Quadratic														
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.5	7.50%	21.17	0.42	1.97%	20.2	1.00%	20.47	0.38	1.85%	18.6	7.00%	17.87	1.02	5.72%
	20	21.3	6.50%				20.3	1.50%				18.3	8.50%			
	20	20.7	3.50%				20.9	4.50%				16.7	16.50%			
	100	104	4.00%	105.33	1.53	1.45%	103	3.00%	102.67	1.53	1.49%	99.9	0.10%	102.63	2.57	2.50%
	100	105	5.00%				101	1.00%				103	3.00%			
	100	107	7.00%				104	4.00%				105	5.00%			
	800	777	2.88%	794.33	24.21	3.05%	811	1.38%	812.67	7.64	0.94%	810	1.25%	804.00	5.57	0.69%
	800	822	2.75%				821	2.63%				803	0.38%			
	800	784	2.00%				806	0.75%				799	0.13%			
Serum	20	21.1	5.50%				20.2	1.00%				18.7	6.50%			
	100	104	4.00%				102	2.00%				101	1.00%			
	800	710	11.25%				790	1.25%				747	6.63%			
Urine	20	21.3	6.50%				21.1	5.50%				17.7	11.50%			
	100	104	4.00%				103	3.00%				101	1.00%			
	800	735	8.13%				776	3.00%				802	0.25%			
		Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/30/11) 19 mix						Between Day			
Blood	20	21.9	9.50%	21.30	0.66	3.08%	20.6	3.00%	20.40	0.28	1.39%		20.23	1.43	7.09%	
	20	21.4	7.00%													
	20	20.6	3.00%				20.2	1.00%								
	100	104	4.00%	105.00	1.00	0.95%	104	4.00%	104.67	2.08	1.99%		104.06	1.96	1.88%	
	100	105	5.00%				107	7.00%								
	100	106	6.00%				103	3.00%								
	800	744	7.00%	777.33	33.01	4.25%	855	6.88%	827.00	34.83	4.21%		803.07	26.95	3.36%	
	800	778	2.75%				788	1.50%								
	800	810	1.25%				838	4.75%								
Serum	20	21.5	7.50%				20.3	1.50%					20.36	1.08	5.29%	
	100	107	7.00%				111	11.00%					105.00	4.06	3.87%	
	800	768	4.00%				767	4.13%					756.40	30.07	3.98%	
Urine	20	20.7	3.50%				20.7	3.50%					20.30	1.48	7.27%	
	100	107	7.00%				107	7.00%					104.40	2.61	2.50%	
	800	744	7.00%				773	3.38%					766.00	26.88	3.51%	

Trifluoperazine

Quadratic

		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
Blood	20	21.7	8.50%	21.43	0.46	2.15%	21.1	5.50%	20.23	0.78	3.84%	18.8	6.00%	18.07	0.81	4.47%
	20	20.9	4.50%				20	0.00%				18.2	9.00%			
	20	21.7	8.50%				19.6	2.00%				17.2	14.00%			
	100	98	2.00%	100.67	2.52	2.50%	99.4	0.60%	100.47	0.92	0.92%	97.8	2.20%	98.10	0.70	0.71%
	100	101	1.00%				101	1.00%				98.9	1.10%			
	100	103	3.00%				101	1.00%				97.6	2.40%			
	800	811	1.38%	812.33	12.06	1.48%	799	0.13%	802.33	7.57	0.94%	790	1.25%	795.00	11.36	1.43%
	800	825	3.13%				811	1.38%				808	1.00%			
	800	801	0.13%				797	0.38%				787	1.63%			
Serum	20	21	5.00%				19.9	0.50%				18.9	5.50%			
	100	98.9	1.10%				102	2.00%				95.4	4.60%			
	800	760	5.00%				795	0.63%				765	4.38%			
Urine	20	21	5.00%				20.6	3.00%				17.4	13.00%			
	100	101	1.00%				100	0.00%				98.3	1.70%			
	800	766	4.25%				807	0.88%				788	1.50%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
Blood	20	20.4	2.00%	19.63	0.68	3.47%	19.8	1.00%	19.83	0.15	0.77%	20	0.00%	19.83	0.21	1.05%
	20	19.1	4.50%				19.7	1.50%				19.9	0.50%			
	20	19.4	3.00%				20	0.00%				19.6	2.00%			
	100	98.6	1.40%	98.93	0.95	0.96%	99.2	0.80%	100.40	1.04	1.04%	101	1.00%	101.67	1.15	1.14%
	100	98.2	1.80%				101	1.00%				101	1.00%			
	100	100	0.00%				101	1.00%				103	3.00%			
	800	793	0.88%	801.00	9.85	1.23%	787	1.63%	789.67	15.18	1.92%	822	2.75%	840.67	23.46	2.79%
	800	798	0.25%				776	3.00%				833	4.13%			
	800	812	1.50%				806	0.75%				867	8.38%			
Serum	20	20.4	2.00%				20	0.00%				20.7	3.50%			
	100	101	1.00%				104	4.00%				104	4.00%			
	800	786	1.75%				794	0.75%				822	2.75%			
Urine	20	20.1	0.50%				20.1	0.50%				19.5	2.50%			
	100	103	3.00%				102	2.00%				103	3.00%			
	800	801	0.13%				792	1.00%				811	1.38%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day		
Blood	20	19.7	1.50%	19.37	0.49	2.55%	20.8	4.00%	20.50	0.42	2.07%		19.83	1.04	5.25%
	20	19.6	2.00%												
	20	18.8	6.00%				20.2	1.00%							
	100	98.6	1.40%	98.57	2.45	2.49%	101	1.00%	100.33	0.58	0.58%		99.89	1.69	1.69%
	100	96.1	3.90%				100	0.00%							
	100	101	1.00%				100	0.00%							
	800	784	2.00%	799.67	15.04	1.88%	839	4.88%	823.67	31.90	3.87%		808.04	21.74	2.69%
	800	814	1.75%				787	1.63%							
	800	801	0.13%				845	5.63%							
Serum	20	19.1	4.50%				19.9	0.50%					19.99	0.73	3.63%
	100	100	0.00%				102	2.00%					100.91	2.85	2.82%
	800	779	2.63%				810	1.25%					788.88	21.10	2.67%
Urine	20	19.1	4.50%				20.5	2.50%					19.79	1.14	5.76%
	100	103	3.00%				102	2.00%					101.54	1.69	1.66%
	800	767	4.13%				791	1.13%					790.38	16.75	2.12%

Ziprasidone

Quadratic

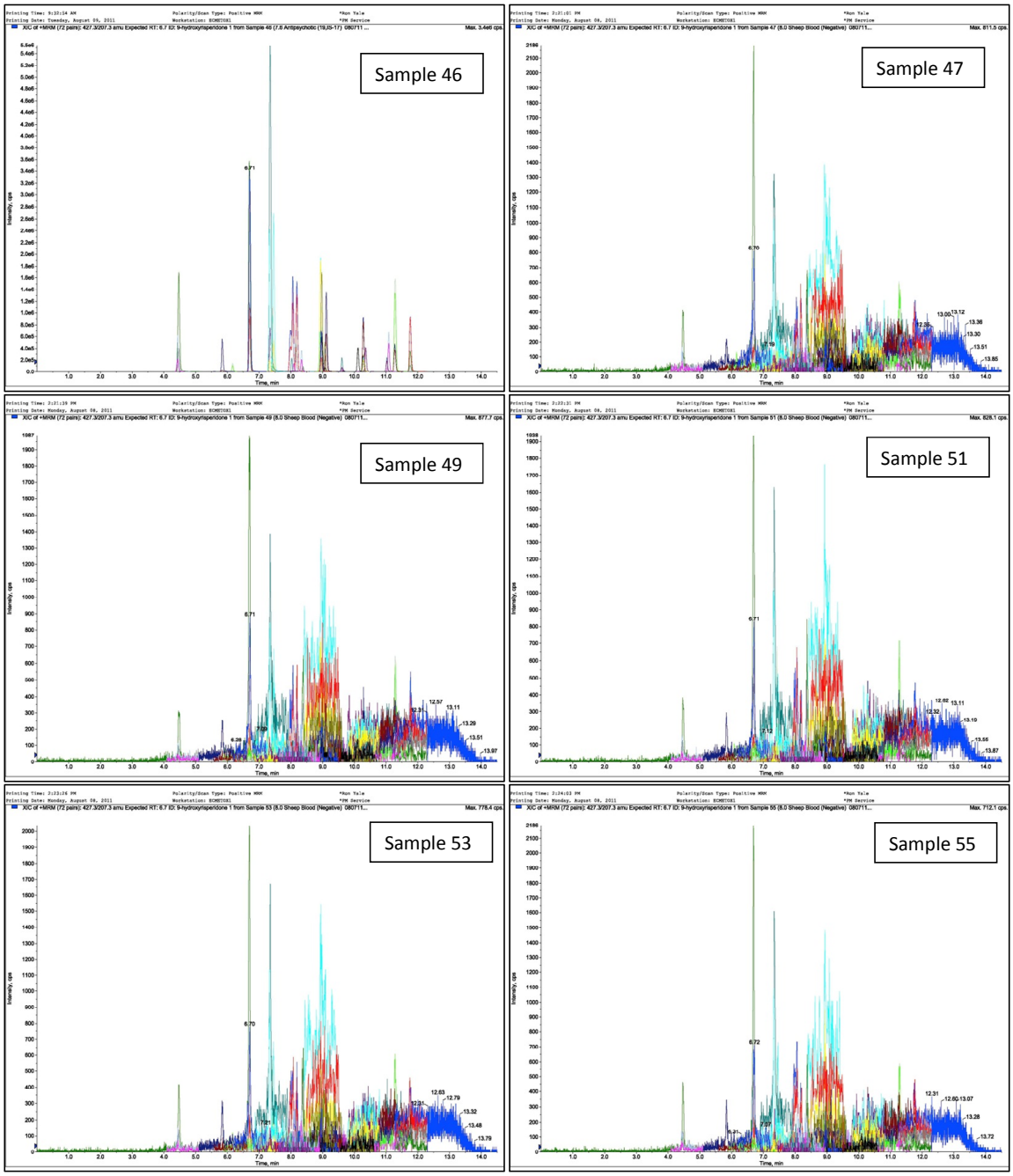
		Day 1 (08/24/11)					Day2 (08/28/11)					Day3 (08/29/11)				
	20	22.2	11.00%				21.2	6.00%				18.4	8.00%			
	20	20.7	3.50%	21.57	0.78	3.60%	21.6	8.00%	21.33	0.23	1.08%	18	10.00%	18.13	0.23	1.27%
	20	21.8	9.00%				21.2	6.00%				18	10.00%			
	100	99.9	0.10%				104	4.00%				96.7	3.30%			
Blood	100	105	5.00%	101.63	2.92	2.87%	108	8.00%	106.33	2.08	1.96%	98.4	1.60%	97.50	0.85	0.88%
	100	100	0.00%				107	7.00%				97.4	2.60%			
	800	813	1.63%				805	0.63%				772	3.50%			
	800	843	5.38%	814.00	28.51	3.50%	810	1.25%	799.33	14.36	1.80%	765	4.38%	766.33	5.13	0.67%
	800	786	1.75%				783	2.13%				762	4.75%			
	20	21.8	9.00%				20.9	4.50%				19	5.00%			
Serum	100	101	1.00%				110	10.00%				99.7	0.30%			
	800	771	3.63%				821	2.63%				764	4.50%			
	20	22	10.00%				20.5	2.50%				17.6	12.00%			
Urine	100	103	3.00%				109	9.00%				97.6	2.40%			
	800	744	7.00%				790	1.25%				773	3.38%			

		Day6 (09/22/11)					Day6 (09/24/11) 19mix Rerun 27th					Day6 (09/24/11) 18 mix				
	20	21.1	5.50%				19.8	1.00%				20.8	4.00%			
	20	18.9	5.50%	20.13	1.12	5.58%	20.1	0.50%	20.07	0.25	1.25%	20	0.00%	20.07	0.70	3.50%
	20	20.4	2.00%				20.3	1.50%				19.4	3.00%			
	100	99.4	0.60%				95.6	4.40%				94.1	5.90%			
Blood	100	102	2.00%	99.37	2.65	2.67%	104	4.00%	100.20	4.26	4.25%	102	2.00%	99.37	4.56	4.59%
	100	96.7	3.30%				101	1.00%				102	2.00%			
	800	785	1.88%				825	3.13%				790	1.25%			
	800	790	1.25%	793.00	9.85	1.24%	860	7.50%	850.67	22.50	2.65%	872	9.00%	861.00	66.19	7.69%
	800	804	0.50%				867	8.38%				921	15.13%			
	20	19.7	1.50%				20.3	1.50%				20.3	1.50%			
Serum	100	97.2	2.80%				107	7.00%				107	7.00%			
	800	811	1.38%				819	2.38%				855	6.88%			
	20	23.2	16.00%				23.1	15.50%				17.3	13.50%			
Urine	100	114	14.00%				127	27.00%				84.8	15.20%			
	800	940	17.50%				890	11.25%				662	17.25%			

		Day6 (09/30/11) 18 mix					Day6 (09/30/11) 19 mix						Between Day		
	20	20.5	2.50%				20.8	4.00%							
	20	19.6	2.00%	20.07	0.45	2.25%			20.35	0.64	3.13%		20.21	1.14	5.62%
	20	20.1	0.50%				19.9	0.50%							
	100	103	3.00%				107	7.00%							
Blood	100	99.6	0.40%	101.53	1.75	1.72%	103	3.00%	105.00	2.00	1.90%		101.37	3.73	3.68%
	100	102	2.00%				105	5.00%							
	800	844	5.50%				925	15.63%							
	800	846	5.75%	834.00	19.08	2.29%	834	4.25%	870.67	48.00	5.51%		823.63	44.45	5.40%
	800	812	1.50%				853	6.63%							
	20	18.5	7.50%				18.7	6.50%					19.90	1.15	5.75%
Serum	100	100	0.00%				104	4.00%					103.24	4.46	4.32%
	800	810	1.25%				818	2.25%					808.63	29.07	3.59%
	20	24.7	23.50%				24.3	21.50%					21.59	2.87	13.28%
Urine	100	125	25.00%				123	23.00%					110.43	14.81	13.42%
	800	947	18.38%				968	21.00%					839.25	112.26	13.38%

APPENDIX D

Carryover Evaluation Data



APPENDIX E

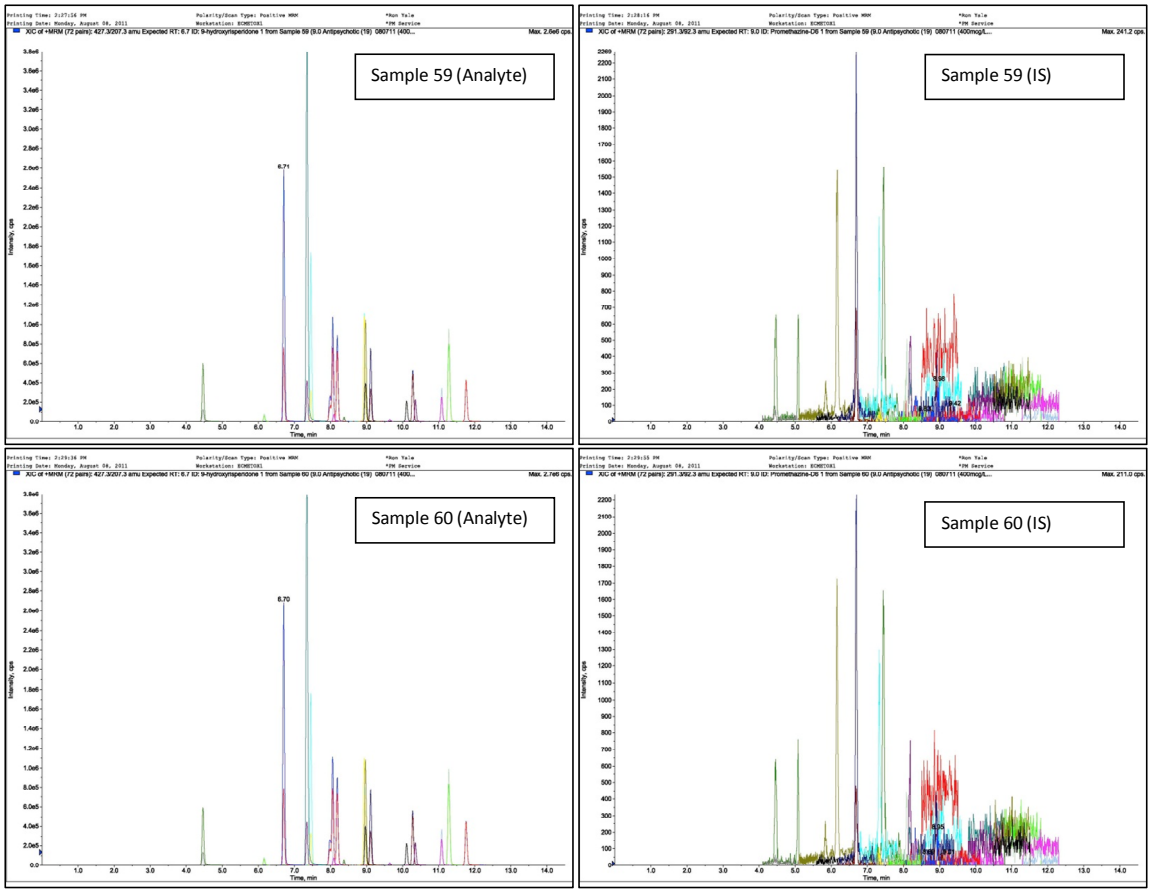
Matrix Effect and Recovery Data

	Promethazine				Quetiapine			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	107.45%	MEC Blood 1373-11 Decomp	103.09%	MEC Blood 1364-11 0256 Fresh	103.11%	MEC Blood 1373-11 Decomp	106.09%
Recovery		62.52%		69.08%		79.16%		78.61%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	107.88%	MEC Blood 0215-11 3951 Decomp	105.20%	MEC Blood 2727-10 0256 Fresh	104.93%	MEC Blood 0215-11 3951 Decomp	105.46%
Recovery		62.06%		82.70%		69.31%		75.22%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	106.84%	Sheep Blood	103.98%	MEC Blood 2648-10 0256 Fresh	100.47%	Sheep Blood	102.02%
Recovery		55.95%		87.25%		76.67%		80.96%
	Olanzapine				**Fluphenazine**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	96.86%	MEC Blood 1373-11 Decomp	89.82%	MEC Blood 1364-11 0256 Fresh	98.76%	MEC Blood 1373-11 Decomp	102.20%
Recovery		14.50%		17.53%		56.68%		59.54%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	97.96%	MEC Blood 0215-11 3951 Decomp	95.26%	MEC Blood 2727-10 0256 Fresh	103.41%	MEC Blood 0215-11 3951 Decomp	103.41%
Recovery		17.26%		16.08%		54.24%		45.54%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	94.39%	Sheep Blood	91.98%	MEC Blood 2648-10 0256 Fresh	104.27%	Sheep Blood	98.65%
Recovery		13.12%		15.19%		51.10%		51.10%
	Risperidone				**Ziprasidone**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	107.48%	MEC Blood 1373-11 Decomp	105.69%	MEC Blood 1364-11 0256 Fresh	105.40%	MEC Blood 1373-11 Decomp	111.45%
Recovery		69.89%		79.85%		74.09%		70.36%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	106.34%	MEC Blood 0215-11 3951 Decomp	106.18%	MEC Blood 2727-10 0256 Fresh	108.17%	MEC Blood 0215-11 3951 Decomp	111.70%
Recovery		67.43%		72.13%		71.33%		56.37%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	107.97%	Sheep Blood	107.48%	MEC Blood 2648-10 0256 Fresh	111.70%	Sheep Blood	110.94%
Recovery		66.42%		75.64%		63.84%		66.98%
	Pipamperone				**Perphenzine**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	97.54%	MEC Blood 1373-11 Decomp	109.06%	MEC Blood 1364-11 0256 Fresh	101.62%	MEC Blood 1373-11 Decomp	23.94%
Recovery		77.95%		77.93%		49.33%		177.36%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	103.64%	MEC Blood 0215-11 3951 Decomp	107.65%	MEC Blood 2727-10 0256 Fresh	29.43%	MEC Blood 0215-11 3951 Decomp	60.44%
Recovery		72.83%		73.13%		57.40%		54.36%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	103.17%	Sheep Blood	105.09%	MEC Blood 2648-10 0256 Fresh	34.35%	Sheep Blood	45.09%
Recovery		72.70%		77.22%		24.56%		66.45%
	9-hydroxyrisperidone				**Haloperidol**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	106.09%	MEC Blood 1373-11 Decomp	106.09%	MEC Blood 1364-11 0256 Fresh	102.46%	MEC Blood 1373-11 Decomp	105.81%
Recovery		70.30%		75.51%		78.07%		75.88%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	106.22%	MEC Blood 0215-11 3951 Decomp	103.66%	MEC Blood 2727-10 0256 Fresh	107.40%	MEC Blood 0215-11 3951 Decomp	104.97%
Recovery		67.83%		72.26%		72.72%		68.06%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	105.55%	Sheep Blood	103.39%	MEC Blood 2648-10 0256 Fresh	106.09%	Sheep Blood	102.46%
Recovery		67.24%		73.39%		68.97%		76.65%

	Buspirone				Mesoridazine			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	106.90%	MEC Blood 1373-11 Decomp	108.03%	MEC Blood 1364-11 0256 Fresh	104.20%	MEC Blood 1373-11 Decomp	107.02%
Recovery		80.93%		78.56%		48.83%		66.02%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	107.13%	MEC Blood 0215-11 3951 Decomp	106.41%	MEC Blood 2727-10 0256 Fresh	103.19%	MEC Blood 0215-11 3951 Decomp	104.73%
Recovery		78.96%		77.43%		47.14%		52.74%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	107.54%	Sheep Blood	108.06%	MEC Blood 2648-10 0256 Fresh	101.97%	Sheep Blood	102.39%
Recovery		73.00%		77.43%		54.90%		58.67%
	Thiotixene				**Aripiprazole**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	103.40%	MEC Blood 1373-11 Decomp	106.56%	MEC Blood 1364-11 0256 Fresh	101.68%	MEC Blood 1373-11 Decomp	107.71%
Recovery		60.59%		70.69%		73.17%		74.94%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	107.30%	MEC Blood 0215-11 3951 Decomp	105.77%	MEC Blood 2727-10 0256 Fresh	101.27%	MEC Blood 0215-11 3951 Decomp	103.26%
Recovery		56.67%		52.67%		72.27%		61.90%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	104.78%	Sheep Blood	102.21%	MEC Blood 2648-10 0256 Fresh	102.15%	Sheep Blood	101.52%
Recovery		60.23%		59.67%		68.17%		70.39%
	Loxapine				**Thioridazine**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	108.35%	MEC Blood 1373-11 Decomp	107.59%	MEC Blood 1364-11 0256 Fresh	104.59%	MEC Blood 1373-11 Decomp	101.29%
Recovery		73.98%		79.65%		55.01%		61.71%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	110.42%	MEC Blood 0215-11 3951 Decomp	106.19%	MEC Blood 2727-10 0256 Fresh	100.90%	MEC Blood 0215-11 3951 Decomp	102.96%
Recovery		72.09%		72.95%		61.38%		52.29%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	110.51%	Sheep Blood	105.84%	MEC Blood 2648-10 0256 Fresh	97.77%	Sheep Blood	99.62%
Recovery		67.46%		77.72%		54.66%		58.56%
	Trifluoperazine				**Clozapine**			
Matrix Effect	MEC Blood 1364-11 0256 Fresh	87.50%	MEC Blood 1373-11 Decomp	99.46%	MEC Blood 1364-11 0256 Fresh	104.33%	MEC Blood 1373-11 Decomp	107.44%
Recovery		58.42%		58.60%		76.13%		78.43%
Matrix Effect	MEC Blood 2727-10 0256 Fresh	97.22%	MEC Blood 0215-11 3951 Decomp	99.06%	MEC Blood 2727-10 0256 Fresh	104.30%	MEC Blood 0215-11 3951 Decomp	104.33%
Recovery		51.34%		47.17%		76.22%		73.81%
Matrix Effect	MEC Blood 2648-10 0256 Fresh	100.34%	Sheep Blood	95.74%	MEC Blood 2648-10 0256 Fresh	101.91%	Sheep Blood	101.63%
Recovery		55.39%		51.29%		73.25%		79.78%
	Chlorpromazine							
Matrix Effect	MEC Blood 1364-11 0256 Fresh	104.22%	MEC Blood 1373-11 Decomp	103.74%				
Recovery		62.73%		65.70%				
Matrix Effect	MEC Blood 2727-10 0256 Fresh	103.27%	MEC Blood 0215-11 3951 Decomp	105.24%				
Recovery		62.25%		54.04%				
Matrix Effect	MEC Blood 2648-10 0256 Fresh	98.84%	Sheep Blood	102.18%				
Recovery		58.64%		57.59%				

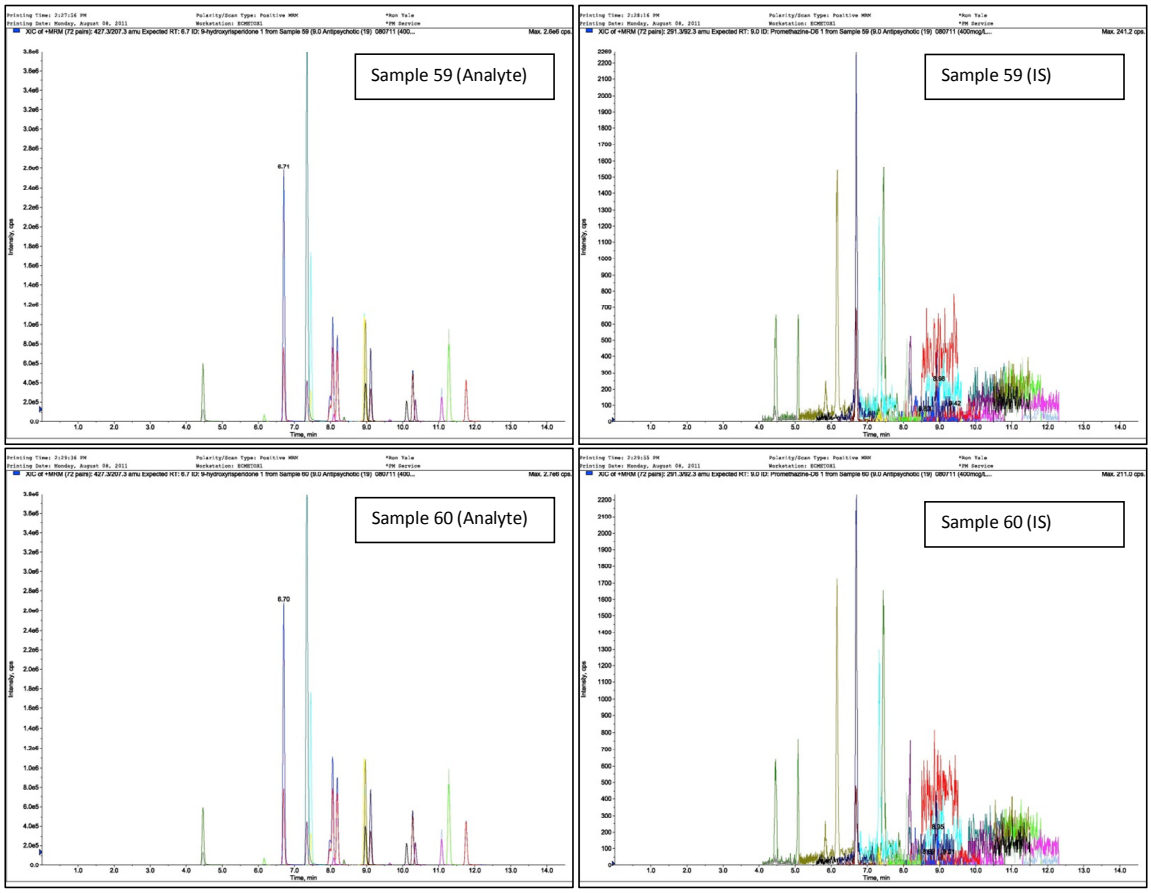
APPENDIX F

Peak Purity and Selectivity

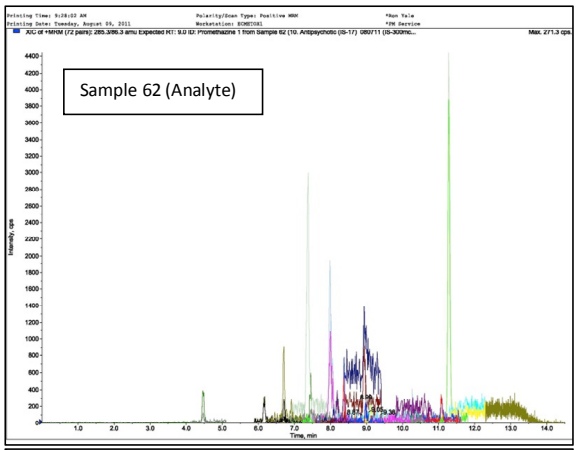


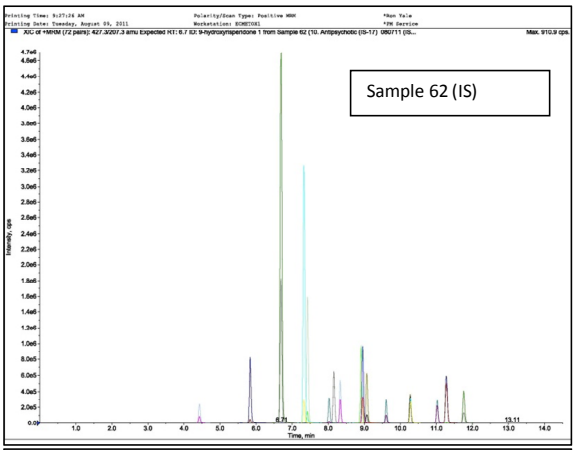
Analyze one zero sample (negative specimen plus I.S.) and one sample containing the analyte(s) but no I.S. to check for the absence of analyte fragmentation ions in the I.S. peak or vice versa.

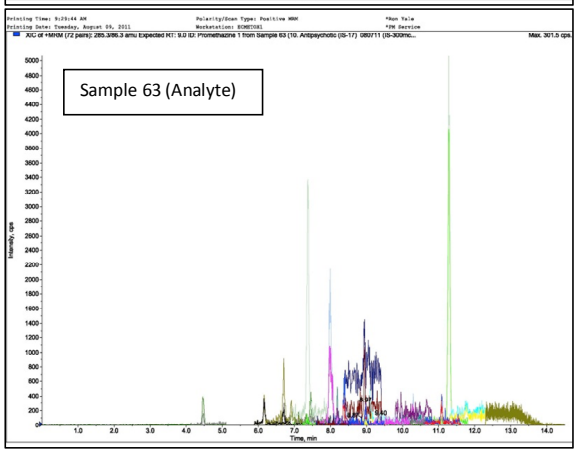
Add **400 µL** of Stock **2.** (For 20 ng, on column) to negative specimen (Sheep Blood).

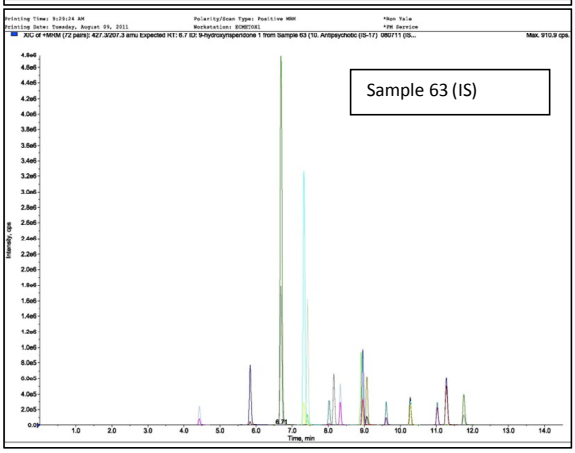


Add **300 µL** of the Standard Solution (15 ng of standard on column) to negative specimen (Sheep Blood).





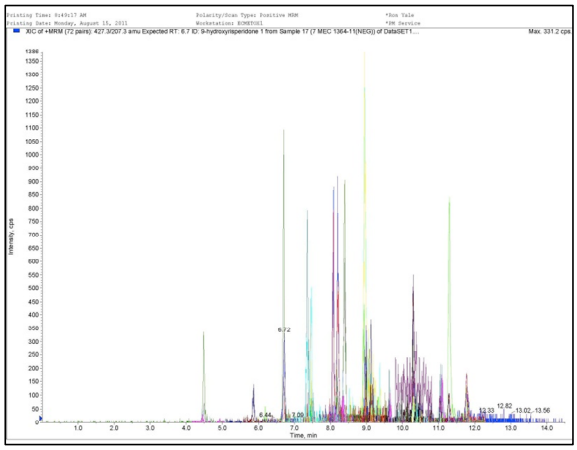




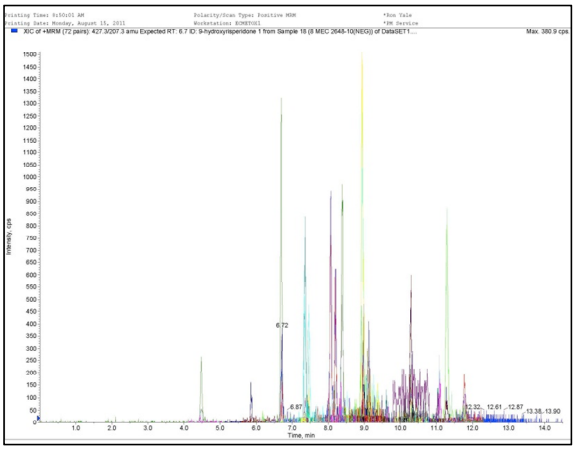
Sample 62				Sample 63			
Analyte Peak Name	**Analyte Peak Area (counts)**	**IS Peak Area (counts)**	**Calculated Concentration (ng/mL)**	**Analyte Peak Name**	**Analyte Peak Area (counts)**	**IS Peak Area (counts)**	**Calculated Concentration (ng/mL)**
9-hydroxyrisperidone 1	3.60E+03	1.76E+07	< 0	9-hydroxyrisperidone 1	4.21E+03	1.78E+07	< 0
Aripiprazole 1	1.73E+02	9.36E+05	0.192	Aripiprazole 1	1.66E+02	9.34E+05	0.17
Buspirone 1	1.53E+03	5.22E+06	< 0	Buspirone 1	1.30E+03	5.19E+06	< 0
Chlorpromazine 1	7.37E+02	1.26E+06	0.0722	Chlorpromazine 1	9.50E+02	1.24E+06	0.124
Clozapine 1	1.24E+03	2.14E+06	< 0	Clozapine 1	1.18E+03	2.17E+06	< 0
Fluphenazine 1	8.91E+02	1.03E+06	< 0	Fluphenazine 1	9.27E+02	1.02E+06	< 0
Haloperidol 1	1.81E+03	3.00E+06	< 0	Haloperidol 1	1.85E+03	2.99E+06	< 0
Loxapine 1	8.02E+02	2.16E+06	< 0	Loxapine 1	1.24E+03	2.17E+06	< 0
Mesoridazine 1	9.68E+03	2.45E+06	< 0	Mesoridazine 1	9.69E+03	2.46E+06	< 0
Olanzapine 1	1.52E+03	8.94E+05	1.1	Olanzapine 1	1.39E+03	8.70E+05	1.08
Perphenzine 1	3.01E+02	1.10E+06	0.261	Perphenzine 1	1.71E+02	1.09E+06	0.186
Pipamperone 1	1.03E+03	2.67E+06	< 0	Pipamperone 1	1.07E+03	2.62E+06	< 0
Promethazine 1	7.71E+02	3.23E+06	0.0321	Promethazine 1	7.22E+02	3.25E+06	0.0259
Quetiapine 1	2.56E+03	1.73E+06	0.86	Quetiapine 1	2.11E+03	1.72E+06	< 0
Risperidone 1	1.14E+04	1.18E+07	< 0	Risperidone 1	1.11E+04	1.17E+07	< 0
Thioridazine 1	1.73E+04	2.45E+06	< 0	Thioridazine 1	1.79E+04	2.46E+06	< 0
Thiotixene 1	1.55E+02	1.03E+06	< 0	Thiotixene 1	5.88E+01	1.02E+06	< 0
Trifluoperazine 1	2.38E+02	1.56E+06	0.00549	Trifluoperazine 1	7.80E+01	1.56E+06	< 0
Ziprasidone 1	4.00E+02	1.09E+06	0.595	Ziprasidone 1	1.50E+02	1.09E+06	0.496

Analyze ten negative specimens of the same matrix from different cases without analyte(s) or intended Internal Standard (I.S.) to check for endogenous peaks that might interfere with the analyte or the I.S.

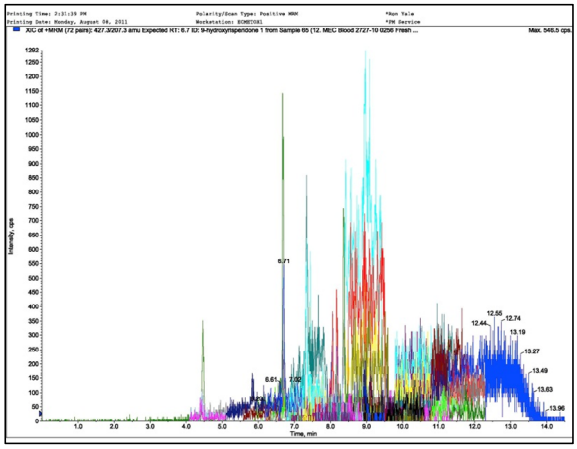
MEC Blood 1364-11 0256 Fresh

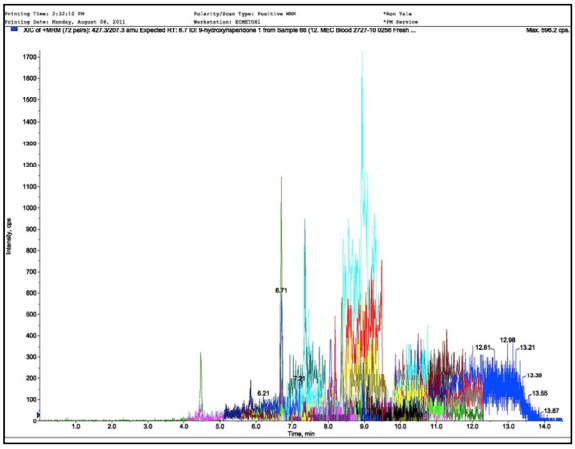


MEC Blood 2648-10 0256 Fresh

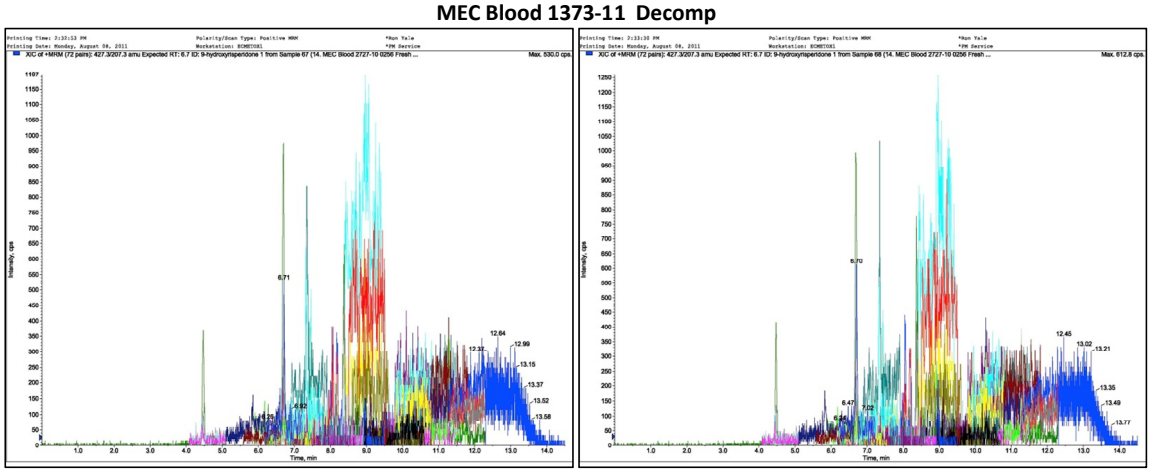


MEC Blood 2727-10 0256 Fresh

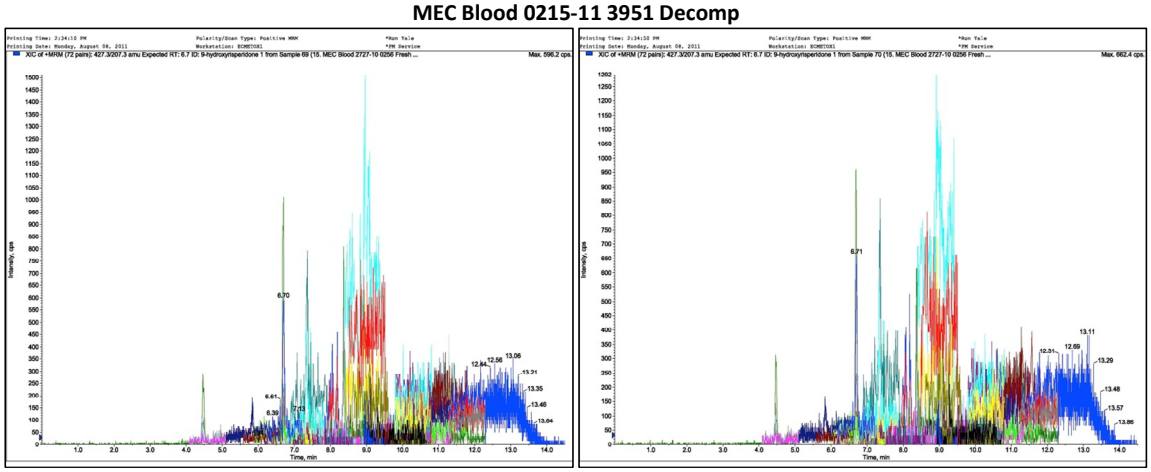




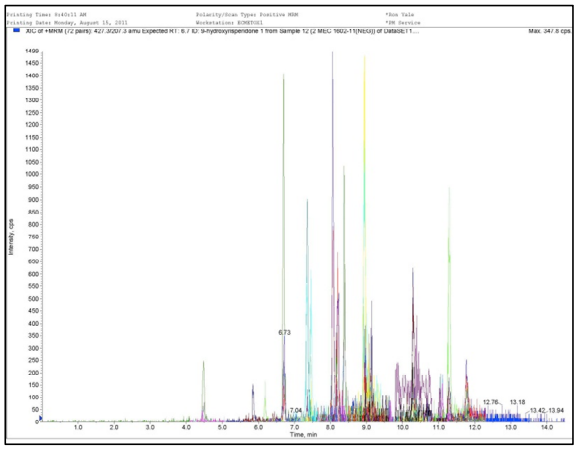
MEC Blood 1373-11 Decomp



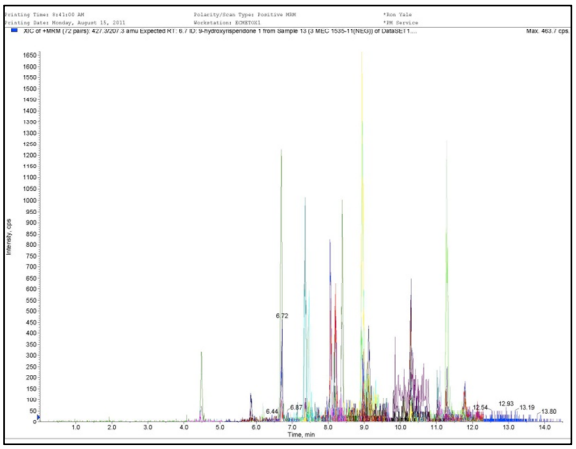
MEC Blood 0215-11 3951 Decomp



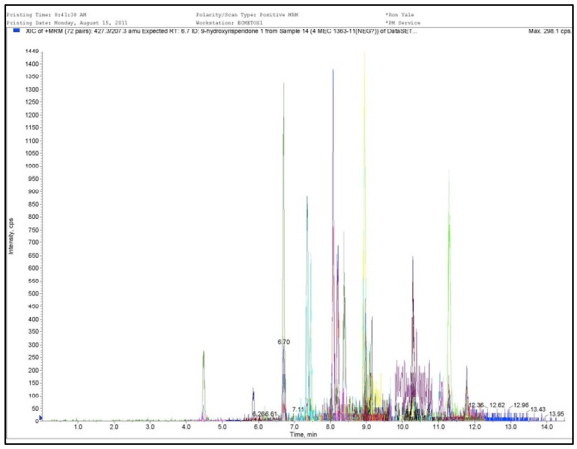
MEC Blood 1602-11 3875 Fresh



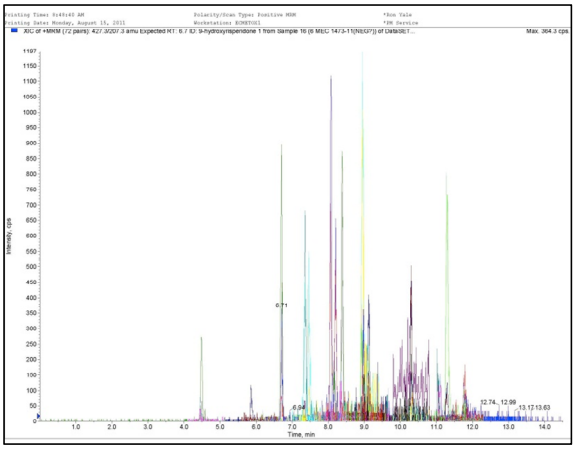
MEC Blood 1532-11 4371 Fresh



MEC Blood 1363-11 Fresh



MEC Blood 1473-11 Fresh



Sheep Blood