DETERMINATION OF ANTIDEPRESSANT AND ANTIPSYCHOTIC DRUG LEVELS IN HUMAN BRAIN TISSUE

By

Melanie Vincenza Ginnis

Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Chemistry

Program

YOUNGSTOWN STATE UNIVERSITY

May, 2001

DETERMINATION OF ANTIDEPRESSANT AND ANTIPSYCHOTIC DRUG LEVELS IN HUMAN BRAIN TISSUE

Melanie Vincenza Ginnis

I hereby release this thesis to the public. I understand this thesis will be housed at the Circulation Desk of the University Library and will be available for public access. I also authorize the University or other individuals to make copies of this thesis as needed for scholarly research.

Signature:

Melanie Vincenza Ginnis 5/4/01

Melanie Vincenza Ginnis, Student — Date

Approvals:

Daryl W. Mincey 5/4/01

Dr. Daryl W. Mincey, Thesis Advisor — Date

Amanda J. Jenkins 5/3/01

Dr. Amanda J. Jenkins, Committee Member — Date

Sherri Lovelace-Cameron 5/4/01

Dr. Sherri Lovelace-Cameron, Committee Member — Date

Peter J. Kasvinsky 5/7/01

Peter J. Kasvinsky, Dean of Graduate Studies — Date

Abstract

In postmortem forensic toxicology, blood and urine specimens are two of the most frequently analyzed specimens to determine the cause of death in individuals. However, these specimens are not always readily available. Therefore, there may be a need to collect alternative specimens for analysis. Brain tissue is an alternative specimen that when present is easy to collect at the time of autopsy. The objective of this research was to develop a method capable of detecting and quantitating common antidepressant and antipsychotic drug concentrations in human brain tissue and indicate the usefulness of brain tissue as a specimen in death investigations.

The method developed involved a liquid-liquid extraction procedure followed by gas chromatographic analysis. The method was validated by determining linearity, limits of detection and quantitation, percent recovery, precision, and accuracy.

All cases used in this research were part of a collaborative study between the Cuyahoga County Coroner's Office in Cleveland, Ohio and Case Western Reserve University Department of Psychiatry in Cleveland, Ohio investigating suicide and depression. The brain tissue specimens were collected by researchers from Case Western Reserve University. Regarding the use of human subjects, the study at Case Western Reserve University was performed in accordance with an approved Institutional Review Board Protocol. In addition, all cases required written familial consent before they were used in this study. Brain frontal cortex and cerebellum from 61 cases were analyzed using this procedure. The data indicated that analysis of brain specimens for antidepressant and antipsychotic drugs may be a valuable adjunct to routine drug testing in more traditional matrices.

Table of Contents

List of Abbreviations

A

B

BAC	blood alcohol concentration
B	mean blank concentration

C

C_M	solute concentration in the mobile phase
C_S	solute concentration in the stationary phase
CV	coefficient of variation
°C	degree Celsius

D

E

ECD	electron capture detector
EPS	extrapyramidal side effects

F

FID	flame ionization detector

G

GC	gas chromatography
GLC	gas-liquid chromatography
GSC	gas-solid chromatography
GC/MS	gas chromatography-mass spectrometry
g	gram

H

HPLC	high performance liquid chromatography
H	plate height

I

IR	infrared

J

K

K	partition coefficient
k’	partition ratio

L

L	length of the column
LOD	limit of detection
LOQ	limit of quantitation

M

MAOI	monoamine oxidase inhibitor
MS	mass spectrometry
M	molar
M^+	parent ion
mL	milliliter
mg	milligram
mm	millimeter
m	meter
m	mass of an ion
min.	minute(s)

N

NPD	nitrogen-phosphorousdetector
ng	nanogram
N	effective plate number
N/A	not applicable
N	normal

O

O.C.	other condition

P

pA*s	relative area

Q

QC	quality control

S

SSRI	serotonin reuptake inhibitor

T

TLC	thin layer chromatography
TCD	thermal conductivity detector
TCA	tricyclic antidepressant
t_R	retention time

t_M	dead time
t'_R	adjusted retention time
U	
UV	ultraviolet
µL	microliter
µm	micrometer
V	
v_R	retention volume
W	
W	width at the base of a peak
X	
Y	
Z	
z	charge on an ion

List of Figures

List of Tables

Chapter One

Introduction

The American Board of Forensic Toxicology states that forensic toxicology is "the study of and practice of the application of toxicology to the purposes of the law.'" The area of forensic toxicology encompasses many fields of interest and serves a multitude of purposes. As an applied science, the main objective of forensic toxicology is to obtain analytical data on chemicals and determine the extent of the involvement of the chemical(s) in a human poisoning. Although forensic toxicology was not considered a science until the early 1800's, it has been around since about 1500 B.C. At this time Egyptian priests discovered and wrote of deadly poisons found in peaches. The start of forensic toxicology as a science began with Orfila. In the early 1800's, he divided poisons into six classes. He also introduced many analytical aspects of forensic toxicology that are still valid to this day. The 1800's also produced the first cases of isolating chemicals in biological samples and the first effective methods of extracting the chemicals from these biological samples. The mid-1900's completely changed the field of forensic toxicology with the invention of sophisticated instrumentation. Infrared (IR) spectroscopy, paper chromatography, and other instruments were used in toxicological analyses as early as the 1940's. Forensic toxicology has changed a great deal since the time of the Egyptian priests, but still remains an important field of science.'

Forensic toxicology investigates potential human poisonings. Often these poisonings are fatal, branching toxicology into the field of postmortem toxicology. This subspecialty is often used to determine or verify the cause of death in an individual. Various biological samples can and should be taken from the individual. Deciding which

samples should be collected usually depends on the nature of the case in question. The most frequently collected sample is blood. It is most often collected from both the heart and peripheral regions of the body to check the distribution of the chemical throughout the body.[2] Another sample frequently collected is urine. It is especially important when looking for drugs of abuse. These drugs are present in urine in microgram quantities, while present in the blood in nanogram quantities.[1] In addition, metabolites occur in greater concentrations in urine than in blood. This is important since many compounds are metabolized, and effective detection involves measuring these metabolites instead of the compound itself.'

Although blood and urine constitute the majority of samples tested by forensic toxicologists, several other samples prove useful. One of these samples is gastric washings and contents, which is important when dealing with acute poisonings. If the original quantity ingested is known, one can estimate the amount of drug absorbed.' The liver may also be collected. The liver is useful since it concentrates many poisons resulting in higher levels than blood samples.[2] Apple and Bandt compared the liver to blood ratios of tricyclic antidepressant concentrations. Their study demonstrated the variance of drug levels in specific regions of the body.[3] Kidneys could also be analyzed to determine the presence of certain chemicals. Their analysis is usually restricted to cases in which metal poisoning is suspected or known.' Finally, the toxicologist may use brain tissue. Brain tissue is an alternative specimen that when present is easy to collect at the time of autopsy. Also, brain tissue analysis may be able to detect drugs that are undetected in blood analysis. The reasoning behind brain tissue research stems from the theory that drugs that have action in the brain will be concentrated there, resulting in easy

detection. It is also assumed that the brain has the property of retaining certain drugs that bind to receptors in the central nervous system.[2] These assumptions however, have not been vigorously tested or proven. Little research has been conducted in this area, which was the motivation behind this project.

Just as the determination of the sample to be analyzed depends on the nature of the case, the method of testing depends on the sample. Many separation, spectrometric, and chromatographic techniques may be employed. Separation techniques are used to extract drugs and chemicals from biological matrices. Some of the separation techniques used include distillation, liquid-liquid extraction, and liquid-solid extraction.' Once the drugs and chemicals are separated they need to be detected and identified. Various instruments are used for this detection.

The first group of detection techniques that is used is spectrometric techniques. The main technique used is Ultra-violet (UV) spectrophotometry. Acidic and basic drugs that are extracted into an aqueous basic or acidic solution can undergo W absorption. Some drugs have characteristic maxima and minima, while others show absorbance only. If specific spectra are not observed further identification tests are needed. Also, since many drugs of the same chemical class have very similar spectra, the W technique is unable to differentiate between them. The drugs can however, be separated into groups based on their characteristic UV absorbance.'

The second group of detection techniques is chromatographic techniques. Thin-layer chromatography (TLC) is a rapid technique used to simultaneously screen a large number of samples. Also, after completion of the analysis the spots may be scraped, the substance reextracted, and confirmed by another analytical technique.[1] Another

chromatographic technique that has been used more often recently is high performance liquid chromatography (HPLC). It is capable of quantifying the drug as well as its metabolites. For example, it has been used to quantify clomipramine, its metabolite, and clothiapine.[4] The final technique used is gas chromatography (GC). Most drugs can be detected by flame ionization (FID), nitrogen-phosphorous (NPD), or electron capture (ECD) detectors. GC is capable of separating parent drugs from their metabolites, as well as drugs that are structurally similar.' Many articles have been written describing GC as the technique used to detect various drugs. One example is research by Drummer et *al*. They described a screening method involving the use of capillary GC with NPD.[5] This method proved to be reliable for analyzing neutral and basic drugs. de la Torre *et al.* described a method for analyzing tricyclic antidepressants and their metabolites. This method involved solid phase extraction and separation by capillary GC-NPD.[6] This method is similar to the method used for this research.

Drugs of Interest

Many classes of drugs can be identified and quantitated in postmortem toxicology. This research investigated only two of these classes, antidepressants and antipsychotics, since their main action is in the brain.

The most common antidepressants can be separated into categories based on their function and structure. The first category is monoamine oxidase inhibitors (MAOIs). MAOIs block the action of the enzyme monoamine oxidase. This enzyme is responsible for the degradation of biogenic monoamines.[7] Some common MAOIs include phenylzine, tranylcypromine, and moclobemide.

The second class is tricyclic antidepressants (TCA). TCAs "inhibit the presynaptic reuptake of the monoamine neurotransmitters norepinephrine and serotonin".[7] TCAs also have affinity for several heterogeneous receptors. TCAs have been reported and quantified in blood and liver.[3] They have also been identified by HPLC in blood, kidney, liver, and gastric contents.[4] Many other articles also report findings of this class of drugs in biological matrices. Some common TCAs include amitriptyline, alompramine, and imipramine. This class of drugs has a wide range of side effects including, dry mouth, dizziness, blurring of vision, and agitation in some cases.[8,9] The main problem with TCAs is that they have a low therapeutic index. Also, overdoses with this type of drug often result in death.[10] Until recently, TCAs were the most commonly prescribed antipsychotics.

The invention of a newer class of antidepressants, selective serotonin reuptake inhibitors (SSRIs), has replaced TCAs as the most commonly prescribed antidepressant. These compounds have a selective effect on the presynaptic reuptake of serotonin.[7] Some

common SSRIs include fluoxetine, paroxetine, and sertraline. SSRIs have less adverse side effects compared to TCAs. Examples of their side effects include headache, sweating, and loss/gain of appetite.[9] The main reason why SSRIs are chosen over TCAs is that rarely are overdoses fatal with this class of drugs. Most overdoses result in either no symptoms or nausea and vomiting.[10]

The final class of antidepressants is novel/atypical antidepressants. These drugs do not have the structural or functional similarities of any of the previously mentioned classes. As with all antidepressants, " the primary mode of action is via a monoamine transmitter reuptake inhibition."[7] Some common novel/atypical antidepressants include trazodone, bupropion, and venlafaxine.

The role of antipsychotics, also known as neuroleptics, in psychotic individuals is to reduce agitation. In non-psychotic individuals, these drugs reduce exploration and the expression of emotion.'' Neuroleptics can be categorized in various ways. One way is by chemical class or potency. In this category, compounds are classified as low, mid, and high potency with high potency compounds reflecting the greatest dopamine binding affinity.[11] Neuroleptics can be classified according to formulation. Many of these drugs are available in oral, intramuscular, and depot formulations. Depending on the clinical case, the drug is distributed in one of these forms and then classified by that form.'' Finally, neuroleptics can be classified according to their pharmacological or clinical profile. In this classification system drugs are classified as conventional or typical drugs if they share the ability to improve positive symptoms of psychoses and cause extrapyramidal side effects (EPS). Drugs classified as atypical show antipsychotic side effects without causing EPS.[11] The majority of antipsychotic drugs act as antagonists at

the dopamine receptor. They are also highly lipophilic and bind to blood proteins.[11] Some common neuroleptics are haloperidol, clozapine, olanzapine, and chlorpromazine.

GC Theory

Chromatography is the separation of a mixture of compounds into separate components through interactions with the mobile and stationary phases. In all separations, the sample is dissolved in a mobile phase. The mobile phase is then forced through a stationary phase that is fixed on a column or a solid surface. The two phases are chosen so that the components in the sample distribute themselves between the mobile and stationary phases at varying degrees. Interactions between the sample and the two phases cause the components of the sample to separate into bands in the mobile phase. The rate at which each component moves through the column depends on the structure of the compound, the chemical structure of the stationary phase, and column temperature. Components that are strongly retained by the stationary phase travel more slowly with the mobile phase. On the other hand, components that are weakly held by the stationary phase travel at a faster rate. Thus, the sample components separate into discrete bands that can be analyzed qualitatively and/or quantitatively.

When the mobile phase is a gas the chromatographic technique is known as gas chromatography (GC).[12] There are two types of gas chromatography: gas-liquid chromatography (GLC) and gas-solid chromatography (GSC). The mobile phase is gas for both GLC and GSC. The gaseous mobile phase is usually referred to as the carrier gas. Carrier gases include nitrogen, argon, and helium, which are all chemically inert. Selecting the proper carrier gas is an important task since the gas affects column and detector performance. In GLC, the stationary phase is a liquid and in GSC, the stationary phase is a solid. GC permits the separation of closely related components of complex mixtures, which would be impossible by other means. Compounds that are suitable for

GC analysis are thermally stable and volatile inorganic and organic compounds. Ten to twenty percent of all known compounds are estimated to be capable of separation by gas chromatography.

The behavior of a sample when in the chromatographic system can be described in multiple ways. One way is the retention behavior, which is indicative of the interactions between the component(s) and the two phases. The retention volume (V_R) is one characteristic that may be obtained. It is the volume of the mobile phase, in GC the volume of gas, required to carry a component band through the entire system and to the detector. Retention time (t_R) is also indicative behavior of a component. The retention time is the time it takes a compound to travel through the column. If all operating conditions are kept the same, a specific compound will always travel through the column at the same specific rate. Due to the specific rate, a compound may be identified by its retention time. Figure 3 shows the retention time of component as seen on a chromatograrn.

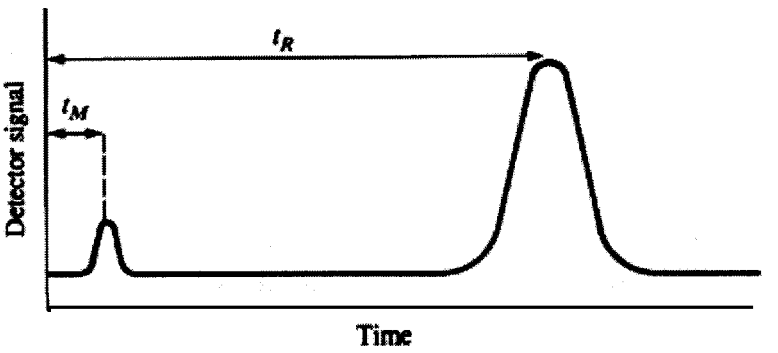


Figure 1: Chromatogram of a one-component mixture[13]

The small peak on the chromatogram that appears before the analyte peak appears is the dead time(t_M). Dead time refers to the time it takes an unretatined species to elute. It is also referred to as the time required for a molecule of the mobile phase to travel through the column. The difference in the retention times ($t_R - t_M$) is referred to as the adjusted retention time (t'_R).

Another way to describe the behavior of a component is by the partition coefficient. When a sample enters the GC, it separates between the two phases. The partition coefficient determines the concentration of the solute in each phase. It is represented by the following equation: $K = C_S/C_M$, where C_S is the solute concentration in the stationary phase and C_M is the concentration of the solute in the mobile phase. When K = 1 the solute is equally distributed between the two phases.

Behavior may also be define by the partition ratio k'. This ratio relates the equilibrium distribution of the compound in the column to the temperature and thermodynamic properties of the column. It is the time the component spends in the stationary phase relative to the time spent in the mobile phase. The partition ratio is found by the equation: $k' = t'_R/t_M$, where t'_R is the adjusted retention time and t_M is the dead time.

Column efficiency is an important aspect of GC since when a solute enters the system its band broadens. The broadening affects the resolution of other solute bands that travel through the system. This efficiency is expressed as an effective plate number (N). It indicates the number of times a solute separates between the two phases while in the column. Plate number can be found from the following equation: $N = L/H$, where L is the length of the column and H is the plate height. Plate Height (H) is the distance a solute

moves while undergoing a separation. Plate number can also be found from the equation: $N = 16(t_R/W)^2$, where t_R is the retention time of the solute and W is the width at the base of the peak.

Gas Chromatograph

There are six basic components in the GC: (1) a carrier gas supply, (2) an injector port, **(3)** a column, (4) an oven, (5) a detector, and (6) a recorder. The GC used in this research was a Hewlett-Packard 6890.

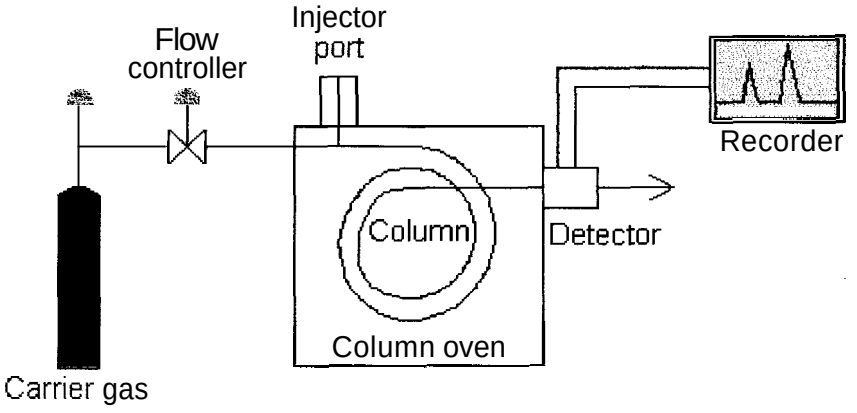


Figure 2: Schematic diagram of a GC[13]

The first component of the system is the carrier gas supply. The role of the carrier gas is to carry the sample through the column. Most often helium, nitrogen, or hydrogen is used. The gas used should be of the highest purity. Impurities in the gas may cause deterioration to the detector and/or column.

The next step in the chromatographic process involves the introduction of the sample into the GC. The sample injection system may consist of a hypodermic syringe. It is injected through a self-sealing rubber septum onto a glass liner where the sample is vaporized and swept onto the column. Another sampling method is an automatic sampler, which duplicates manual sample measurements and injection. Auto samplers are machine

reproducible and more precise than manual injections. An autosampler was used in this research. Headspace sampling, purge and trap, and pyrolysis are a few more methods for GC sampling.

Next, the vaporized molecules enter the column. There are two different types of columns available: packed and capillary. Packed columns are constructed of stainless steel, nickel, or glass tubing. Their internal diameters range from 1.6-9.5 mm, with a length of 3 m. These columns are packed with an inert support that has pore diameters of 2-9 μm. Capillary columns are constructed of fused silica and have an internal diameter of 1mm or less. (Figures 2 and **3)** A thin film of a high molecular weight thermally stable polymer is coated onto the inner wall of the column. The polymer coating is the stationary phase. An **RTx** 50 capillary column was used in the research.

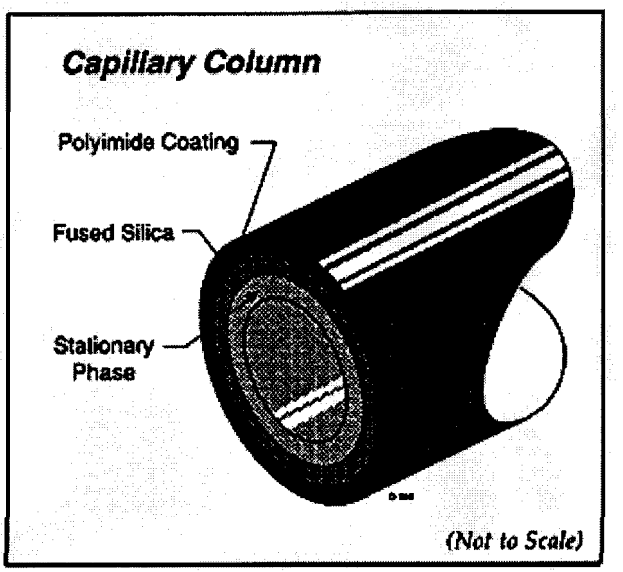


Figure 3: Capillary column[13]

The oven of the chromatograph is the housing for the column. The column is coiled and hung in a basket inside the oven. The oven must be able to be rapidly heated and cooled. Most are made of stainless steel.

The next part of the system is the detector, which is located at the exit of the separation column. The purpose of the detector is to sense the presence of individual components as they leave the column. The detector monitors the carrier gas as it exits the column and generates a signal based on the variation in its composition due to eluted components. There are several types of detectors that are commonly used such as, flame ionization (FID), electron capture (ECD), nitrogen-phosphorous (NPD), and thermal conductivity (TCD). The choice of a detector depends on the selectivity required and the analytes being studied. This research involved analysis with GC-NPD. The NPD differs from other detectors in that there is a small amount of ionization of carbon-hydrogen bonds and an increased ionization of phosphorous and nitrogen containing compounds. Since the majority of antidepressant and antipsychotic drugs contain nitrogen the NPD was the best detector for analysis. The mechanism of the detector is not completely known, but a majority is. The column effluent is mixed with hydrogen and is ignited as it passes through a flame tip. The hot gas then flows around a heated bead, which is made of rubidium silicate, and a plasma is formed. The combustion products of the nitrogen and phosphorous containing products react with the metal ions. This causes the production of thermoionic electrons that are attracted to the collector. The ions hit the collector and generate a signal.

Once the signal is produced, the data is sent to a computer, integrator, or recorder and a chromatogram is produced. Various other types of information such as retention

times, peak areas, peak widths, and area percent are also generated. An ideal chromatogram is obtained when all of the analytes in a mixture are separated with baseline resolution in minimum time. The time and size of a peak are the most important features on a chromatogram that allow for quantitative and qualitative analysis. The size of the peak in terms of the area under the peak corresponds to the concentration of the component. A larger area is obtained with the increase in the concentration of a component. As stated previously the retention time of a component can identify it. In order to correctly identify or quantitate a compound(s) a standard solution of the compound(s) with a known concentration must also be analyzed and its retention time and peak area determined. The sample and the standard can then be compared to determine the identity and quantity of the sample compound. If two compounds or peaks have the same retention time, an accurate identification is impossible. Therefore, it is desirable to have no peak overlap or coelution occur.

Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GCMS) is a coupled technique, combining the techniques of both instruments. This makes it a very powerful technique with increased sensitivity for identification and confirmation of results. Figure 4 shows a schematic diagram of a mass spectrometer.

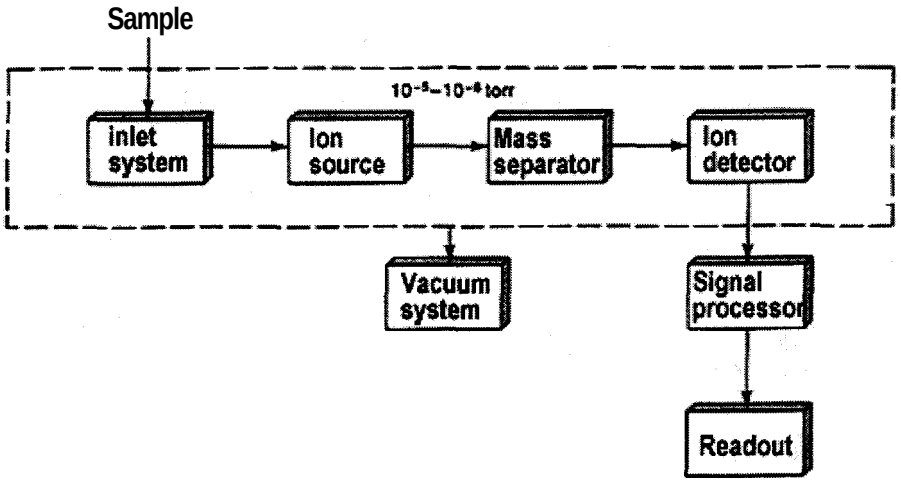


Figure 4: Components of a mass spectrometer[13]

In GCMS, the compound passes through the GC and is then converted into ions in the mass spectrometer. In MS, charged particles are produced that consist of a parent ion (M^+) and ion fragments of the original molecule. These fragments are sorted based on their masslcharge ratio, represented as m/z, where m is the mass of the ion and z is the charge on the ion, Most of the ions have a charge based on the loss of one electron. Once a compound has traveled through the mass spectrometer, a mass spectrum is produced. It is a record of the numbers of different kinds of ions produced by the compound. Each

molecule has its own characteristic spectrum, even isomers. Most mass spectrometers are equipped with a computer that has software containing a library of spectra for an unlimited amount of molecules. The spectrum obtained from analysis can be compared to the spectra within the library and a match can be made. This can provide elemental composition of the molecular and fragment ions. Combining the data and results achieved from mass spectrometry with those of another technique can usually provide definite identification of a compound.

Chapter Two

Project Information

This research project considered two different aspects in determining the presence of antidepressant and antipsychotic drugs in human brain tissue. The first aspect of the project was to develop an assay capable of quantitating and identifying these types of drugs in the specimens. Once the assay was developed it was validated. The validation parameters included limit of detection (LODs), limit of quantitation (LOQs), percent recovery, and linearity. These parameters were conducted for all twenty-three analytes studied. The accuracy and precision validation parameters were conducted for a quality control that contained six drugs. The twenty-three analytes validated for the assay were as follows:

Antidepressants		Antipsychotics
Amitriptyline	**Desipramine**	**Chlorpromazine**
Nortriptyline	**Fluoxetine**	**Clozapine**
Amoxapine	**Mirtazapine**	**Haloperidol**
Bupropion	**Sertraline**	**Hydroxyzine**
Citalopram	**Norsertraline**	**Loxapine**
Doxepin	**Trazodone**	**Olanzapine**
Nordoxepin	**Venlafaxine**	**Thioridazine**
Imipramine	**Norvenlafaxine**	

Overall, 19 antidepressant and antipsychotic drugs were analyzed as well as four metabolites. The metabolites for amitriptyline (nortriptyline), imipramine (desimprarnine), sertraline (norsertraline), and venlafaxine (norvenlafaxine) were also analyzed.

The second aspect of the research was to use the assay developed to analyze specific cases available from Office of the Cuyahoga County Coroner (Toxicology laboratory) Cleveland, Ohio. Frontal cortex and cerebellum were collected and stored at -20 °C then analyzed for each case. These cases were analyzed using the assay and positive results were confirmed in one matrix by GC/MS. Sixty-one cases were analyzed, with 24 cases identified as controls. A control is a case where the individual was psychiatrically and neurologically normal, and had no diagnosed psychoactive substance use disorder. The drug concentrations found in the brain were compared to the drug concentrations found in the brain for each case. Table 1 indicates the demographic data of the individuals used in this study.

,CASE#	AGE	RACE	SEX	CAUSE OF DEATH	MANNER
1*	46	B	M	Probable hypertensive coronary sclerotic heart disease with acute myocardial necrosis and cardiomegaly.	NATURAL
2*	62	W	M	Cardiomyopathy.	NATURAL
3	81	W	M	Drowning.	SUICIDE
4*	69	B	M	Hypertrophic, ischemic, and congestive cardiomyopathy with severe coronary atherosclerosis. O.C. Diabetes mellitus.	NATURAL
5*	44	W	M	Hemopericardium with cardiac tamponade. Due to: Spontaneous inrapericardial rupture of dissecting aortic aneurysm.	NATURAL
6*	20	B	M	Hypertrophic cardiomyopathy, etiology undetermined, with cardiomegaly and congestive heart failure.	NATURAL
7	48	W	M	Asphyxia by carbon monoxide (inhalation of auto exhaust).	SUICIDE
8*	36	B	F	Chronic asthmatic bronchitis with acute bronchial asthma. O.C. Hypertensive cardiovascular disease with cardiomegaly.	NATURAL
9*	43	W	M	Organizing & acute bilateral occluding pulmonary thromboemboli. Organizing & acute phlebothromboses of bilateral lower extremities. Due to: Obesity.	NATURAL
10*	67	W	M	Atherosclerotic cardiovascular disease.	NATURAL
11*	37	W	M	Acute hemorrhagic pancreatitis. Due to: Choledocholithiasis.	NATURAL
12*	51	B	F	Hypertrophic cardiomyopathy with severe coronary atherosclerosis, remote myocardial infarct, cardiomegaly and congestive hgart failure.	NATURAL
13*	50	W	M	Atherosclerotic heart disease with myocardial infarcts and cardiomegaly.	NATURAL

Table 1: Demographic Case Data (* = control)

CASE#	AGE	RACE	SEX	CAUSE OF DEATH	MANNER
14*	80	W	F	Hypertensive coronary sclerotic heart disease w/remote myocardial infarct.	NATURAL
15	35	B	M	Asphyxia by carbon monoxide (inhalation of auto exhaust).	ACCIDENTAL
16	62	W	M	Acute bronchopneumonia. Due to: Coronary sclerotic heart disease.	NATURAL
17*	42	B	M	Dissecting aortic aneurysm with rupture into pericardial cavity and cardiac tamponade.	NATURAL
18	36	W	M	Hypertensive cardiovascular disease. O.C. Morbid obesity.	NATURAL
19*	38	B	F	Hypertensive cardiomyopathy. O. C. Diabetes mellitus. Bronchial asthma.	NATURAL
20	70	W	M	Atherosclerotic cardiovascular disease with remote myocardial infarcts, focal acute myocardial ischemia, and cardiomegaly.	NATURAL
21	35	W	M	Hypertrophic and ischemic cardiomyopathy.	NATURAL
22	82	W	M	Asphyxia by carbon monoxide (inhalation of auto exhaust).	SUICIDE
23	42	W	M	Dilated cardiomyopathy with coronary sclerotic heart disease, myocardial fibrosis, myocardial infarcts and cardiomegaly.	NATURAL
24	29	W	M	Hanging.	SUICIDE
25	34	B	M	Dilated cardiomyopathy. O. C. Acute intoxication by cocaine.	ACCIDENTAL

Table 1 cont.: Demographic Case Data (* = control)

CASE #	AGE	RACE	SEX	CAUSE OF DEATH	MANNER
26*	46	W	F	Atherosclerotic heart disease.	NATURAL
27*	69	B	F	Coronary sclerotic heart disease with transmural myocardial infarct and myocardial rupture. O.C. Diabetes mellitus.	NATURAL
28	78	B	M	Gunshot wound of head with perforations of skull and brain.	SUICIDE
29	78	Asian	M	Hanging.	SUICIDE
30*	74	B	F	O.C. Diabetes Mellitus.	NATURAL
31	36	B	M	Blunt impacts to head and trunk with visceral, skeletal and soft tissue injuries.	ACCIDENTAL
32	58	C	M	O.C. Non-insulin dependent diabetes mellitus.	NATURAL
33	44	W	M	Shotgun wound of chest with perforations of heart, aorta, and right lung.	SUICIDE
34*	35	B	M	Hypertensive and hypertrophic cardiomyopathy. O.C. Morbid obesity.	NATURAL
35*	86	W	F	Coronary sclerotic heart disease with myocardial fibrosis and cardiomegaly.	NATURAL
36	30	W	F	Hanging.	SUICIDE
37*	55	B	M	Hypertensive atherosclerotic cardiovascular disease. O.C. Diabetes mellitus.	NATURAL

Table 1 cont.: Demographic Case Data (* = control)

CASE #	AGE	RACE	SEX	CAUSE OF DEATH	MANNER
38	58	**W**	**M**	Gunshot wound of head with perforations of skull and brain.	SUICIDE
39*	**37**	**W**	**F**	Viral myocarditis.	NATURAL
40	78	**W**	**M**	Severe coronary sclerotic heart disease, probable hypertensive, with remote	NATURAL
				myocardial infarct, acute microinfarct and cardiomegaly. O.C. Severe chronic	
				obstruct. pulmonary disease **w/acute** bronchiolitis & early bronchopneumonia	
41	30	B	**M**	Coronary sclerotic heart disease with cardiomegaly. O.C. Remote drug abuse	ACCIDENTAL
42	57	**W**	**F**	Hypertrophic cardiomyopathy, probably hypertensive with coronary sclerotic	NATURAL
				heart disease, myocardial fibrosis, and cardiomegaly.	
43*	41	B	**F**	Atherosclerotic heart disease with thrombotic occlusion of right coronary	NATURAL
				and acute myocardial infarct.	
44	29	**W**	F	Acute bronchopneumonia and massive liver necrosis with hepatic failure.	ACCIDENTAL
				Due to: Acute intoxication by acetaminophen and combined effects of	
				hydrocodone and meperidine. O.C. Chronic ethanol abuse.	
45	81	**W**	**F**	**Acute** bronchopneumonia. Due to: Atherosclerotic heart disease.	NATURAL
				O.C. Ischemic bowel disease with infarction.	
46	**64**	B	**M**	Gunshot wound of head with perforations of skull and brain.	SUICIDE
47	**74**	**W**	**M**	Ruptured arteriosclerotic abdominal aortic aneurysm with exsanguination.	NATURAL
48*	57	**W**	**M**	Lobar pneumonia. O.C. Atherosclerotic heart disease with congestive failure.	NATURAL
49	59	**W**	F	Acute intoxication by hydrocodone.	SUICIDE

Table 1 cont.: Demographic Case Data (* = control)

CASE #	AGE	RACE	SEX	CAUSE OF DEATH	MANNER
50	32	**W**	**F**	Intraoral gunshot wound with perforations of skull and brain.	SUICIDE
51	33	**W**	**M**	Gunshot wound of head with perforations of skull and brain.	SUICIDE
52	80	**W**	**M**	Gunshot wound of head with perforations of skull and brain.	SUICIDE
53	24	**B**	**M**	Acute intoxication by olanzapine.	SUICIDE
54	42	W	**M**	Hypertrophic cardiomyopathy, probably hypertensive and atherosclerotic	NATURAL
				cardiovascular disease. O.C. Bullous pulmonary emphysema, severe.	
55	53	W	F	Atherosclerotic heart disease, probably hypertensive with cardiomegaly.	NATURAL
				O.C. Diabetes mellitus.	
56*	44	**B**	**F**	Hypertensive cardiomyopathy and atherosclerotic cardiovascular disease.	NATURAL
				O.C. Diabetes mellitus and obesity.	
57	41	W	**M**	Acute intoxication by combined effects of ethanol and oxycodone.	NATURAL
58	40	W	**M**	Hanging.	SUICIDE
59	47	W	**M**	Asphyxia by carbon monoxide (inhalation of auto exhaust).	SUICIDE
60	63	**B**	**M**	Hypertensive, hypertrophic and ischemic cardiomyopathy with remote and	NATURAL
				organizing myocardial infarcts.	
61	61	W	**M**	Atherosclerotic cardiovascular disease.	NATURAL

Table 1 cont.: Demographic Case Data (* ≐ control)

Chapter Three

Literature Review

While a large amount of research has been conducted on determining and identifying chemicals in blood, urine, and various other biological matrices, little research has been conducted on the brain.

Experiments with Alcohol

The literature does offer some articles on the determination of ethanol in brain samples. In a study by Backer ***et al.***, ethanol concentrations were compared in various biological specimens, including brain tissue. This study was conducted to determine the correlation between blood alcohol concentration (BAC) and other body fluids and tissues. This is important since blood is not readily available in all cases. In Backer's study the brain samples were frontal lobe tissues. This study determined the average brain fluid to blood alcohol ratios to be 0.86. From the various sample ratios, the study determined a formula to calculate the BAC from the alcohol concentration in another fluid or tissue.[14]

A more recent study conducted by Moore et *al.* compared ethanol concentrations in the occipital lobe and cerebellum. This study investigated the regional distribution of ethanol in the brain and compared the values obtained to the blood alcohol concentration. The study determined the average occipital and cerebellum/blood alcohol ratios to be 0.8 and 0.7 respectively. These values did not differ much from each other or from previously reported values. The authors concluded, "as long as samples which consist primarily of gray matter are used for analysis, brain ethanol concentrations can be used judiciously to give an approximation of blood ethanol levels".[15] Gray matter is areas of the brain that are more vascular than white matter and have a lower fat content. Examples

of gray matter are the frontal cortex and cerebellum, which were used in the research presented. Their research indicated that the area of the brain did not have a significant influence on the ethanol concentration.

Animal Studies

The literature also contains studies that determine various compounds in rat brain. One investigation performed was the determination of three novel anxiolytic agents and their common metabolite in rat plasma and brain by HPLC. Buspirone, ipsapirone, and gepirone are known to be concentrated in the brain from previous animal studies. Prior studies conducted on these compounds focused only on one parent compound by radioimmunoassay or GC-MS. The metabolite had been analyzed separately by ECD and GC-MS. A simultaneous determination with HPLC had never been made. The researchers concluded that the HPLC simultaneous method was an efficient way to identify the three drugs and their common metabolite in rat brain. Their method also established a quantitative relationship between brain concentrations and central effects.[16]

Another study quantified buspirone levels in rat brain by GC-NPD. The paper described a rapid, sensitive assay to quantify the drug levels, versus more time consuming methods. Calibration curves were constructed that were reproducible and from these results an assay was developed. This assay is now used to measure buspirone levels in the brains of rats. Lai ***et al.*** concluded from the results that they developed an analytical procedure that was linear, sensitive and reproducible.[17] The result was that GC-NPD was an applicable technique to measure buspirone levels in the brain of rats.

Drug Studies

Various studies have been performed determining and identifying drugs in postmortem brain tissue. These studies, however, are limited in scope. They draw no correlation between brain tissue concentrations and concentrations in other matrices. Most do not show simultaneous determinations of many drugs; they are limited to only a few. In addition, the research conducted investigated drugs other than antidepressants and antipsychotics.

The literature does offer one example of the simultaneous determination of several drugs in brain tissue. The study determined eleven antidepressant drugs and their metabolites from human postmortem samples, as well as, other matrices. The samples were analyzed by GC-NPD. The highest recoveries were found in the blood (94.9%) with the narrowest range, while the recoveries from brain were approximately (85%).[18] Although this research indicates that antidepressants can be detected in brain, it does not give any significance to the resulting recoveries.

Another example is the simultaneous determination of Haloperidol and its metabolite by Igarashi ***et al.*** This study established a method to detect these drugs in the plasma and brain of schizophrenic patients who had been treated with the drug. The brain of only one patient was analyzed for both of the drugs. Haloperidol and its metabolite were detected in the brain of this patient.[19] Although the drugs were detected in the brain, the main focus of the research was the detection of the drugs in the plasma of the patients.

Experimental

Materials and Methods

The research method for this study included preparation, separation, identification, quantitation, and confirmation of drugs in brain specimens. The specimens were collected from autopsies routinely performed at the Cuyahoga County Coroner's Office. Blood analysis was performed by the Toxicology Laboratory as part of routine drug testing. The brain specimens from the cerebellum and frontal cortex were collected by personnel Case Western Reserve University Department of Psychiatry as a part of a study investigating suicide and depression.

A list of the reagents used in the assay includes the following: sodium chloride, sodium hydroxide, ammonium chloride, concentrated ammonium hydroxide, concentrated sulfuric acid, and concentrated hydrochloric acid which were purchased from Mallinkroft Chemical Co. (Paris, KY), ethyl acetate, hexane, and isopropanol which were purchased from American Burdick and Jackson Laboratories Inc. (Muskegon, MI), and TRIZMA BASE which was purchased from Sigma Chemical Co. (St. Louis MO). Sodium chloride saturated deionized water was prepared by adding sodium chloride to 1.0 L of deionized water until the solution was supersaturated. To prepare 6 N sodium hydroxide, 240 g of sodium hydroxide was added to a 1.0 L graduated cylinder half filled with deionized water. Once dissolved it was filled to the mark with deionized water. Ammonium chloride/ammonium hydroxide buffer (pH 9.2) was prepared by adding ammonium chloride to 1.0 L of deionized water until the solution was supersaturated. Then concentrated ammonium hydroxide was added to the solution with a 5.0 mL serological pipette until a pH of 9.2 was obtained. To prepare 0.5 N sulfuric acid, 13.9

mL of concentrated sulfuric acid were added to a 1.0 L volumetric flask half filled with deionized water, then diluted to the mark with deionized water. Tris buffer (1.2 M, pH 9.3) was prepared by adding145.32 g of TRIZMA BASE to a 1.0 L volumetric flask half filled with deionized water. Once dissolved the flask is filled to the mark with deionized water. The pH was adjusted to 9.2 with concentrated hydrochloric acid. Hexane/isopropanol (9:1) was prepared by adding100 mL of isopropanol to a 1.0 L graduated cylinder and filling to the mark with 900 mL of hexane. To prepare 0.1% hydrochloric acid/methanol 100 µL of concentrated hydrochloric acid was added to a 100 mL volumetric flask and diluted to the mark with methanol.

As well as preparing reagent solutions, standard solutions were also prepared. A 5000 ng/mL solution of promazine was used as the internal standard. It was prepared by pipetting 2.5 mL of 1000 mg/L promazine stock standard solution into a 500 mL volumetric flask partially filled with deionized water and transferring to an autopipettor. This solution is stable for one month. Seven calibrators (1000 ng/mL) that contained different antidepressant and antipsychotic drugs were made by pipetting 50 µL of base standard solutions into 5 mL of drug free brain homogenate (30g brain tissue + 150 mL deionized water) in separate culture tubes. High and low positive controls (1000 ng/mL and 250 ng/mL), QCs, were made by pipetting 50 µL of each QC separately into 5 mL of drug free brain homogenate in culture tubes. A negative control was prepared by pipetting 5 mL of drug free brain homogenate into a culture tube.

Liquid-liquid Extraction Assay

Specimens were prepared for analysis by placing 6 g of brain tissue and 10 mL of deionized water into a commercial blender. The mixture was blended for approximately 25 seconds and transferred to a 35 mL screw-capped glass culture tube, with a Teflon lined screw cap.

The first step of the procedure was to combine in a series of 35 mL culture tubes (a) 5 mL homogenate, calibrator, or control, (b) 1.0 mL (5000 ng/mL) promazine internal standard solution, (c) 2 mL sodium chloride saturated deionized water, (d) 100 µL (6M) sodium hydroxide, (e) 2 mL (pH 9.2) ammonium chloride/ammonium hydroxide buffer **(f)** 10 mL of ethyl acetate. All tubes were capped and rotated for 20 minutes, then centrifuged for 10 minutes at 3000 rpms. The upper (organic) layer was recovered and transferred to a clean 16 mL culture tube, the lower layer was discarded. Next 2 mL of 1.0 M sulfuric acid was added to the tube. All tubes were capped with Teflon lined caps, rotated for 20 minutes, and then centrifuged for 5 minutes at 3000 rpms. The lower aqueous layer was recovered by inserting a 9 inch disposable pipette with bulb through the upper organic layer, and aspirating the acid layer into the pipette. The acid layer was then transferred to a clean 16 mL culture tube. Next (a) 4 mL of Tris buffer (pH 9.3) and (b) 5 mL of hexane/isopropanol solution (9:1) were added to each tube. The tubes were capped with Teflon lined caps, rotated for 20 minutes, and centrifuged for 5 minutes at 3000 rpms. Upper (organic) layer was transferred into a clean 15 mL dispotube with a 5 mL serological pipette, and 1 drop of hydrochloric acid in methanol (0.1%) was added. All tubes were evaporated to dryness at 35°C in TURBO-VAP® (Zymark Corp.) Residue was reconstituted with 100 µL of methanol,

vortexed briefly, and transferred to an automatic injection vial that contained a 300 µL glass vial. All vials were sealed with an aluminum cap that contained a Teflon/rubber septum and the analyzed on the gas chromatograph.

The gas chromatograph used for this assay was a Hewlett-Packard 6890 GC equipped with a nitrogen-phosphorous detector and an RTx-50 (30 m x 0.32 mm I.D., 0.25 film thickness) capillary column. Initial oven temperature of 130°C was maintained for 2.0 minutes, then increased to 300°C at a rate of 15°C/min. Final oven temperature 300°C was maintained for 20 minutes. The injection port and nitrogen-phosphorous detector temperatures were 240°C and 300°C respectively. Helium was used as the carrier gas at a flow rate of 2.0 mL/min. A HP 5973 MSD GC/MS was used to confirm positive GC results that were not confirmed by blood analysis.

Assay Validation Parameters

Linearity was determined by extracting drug free brain homogenate with increasing concentrations of each calibrator over the range 0-3000 ng/mL. The LOD was determined by adjusting the attenuation on the GC so that the background peaks (noise) of the chromatogram were integrated. The mean ratio value of these peaks was then determined and converted to a mean blank concentration (B). The LOD for each drug was determined as 3B. The LOQ was determined by the equation 10B or approximately 3(LOD). The extraction recovery was determined by the ratio of the extract peak area to the corresponding area of the unextracted methanolic standard and reported as a percentage.

The validation parameters accuracy and precision were determined by assaying the following six drugs: fluvoxamine, amitriptyline, chlorpromazine, sertraline, loxapine, and thioridazine. Within-day precision was calculated from repeated analysis (N=9) of positive controls at two concentrations (1000 ng/mL and 250 ng/mL). It was reported as coefficient of variation (%CV). Between-day precision was calculated from data analysis on five separate days at 1000 ng/mL and 250 ng/mL, reported as %CV. Accuracy was determined by running nine positive controls at 250 ng/mL and comparing to experimentally determined target concentration.

Chapter Four

Results

Assay Characteristics

The upper limit of linearity was 3000 ng/mL for all drugs except three. It was 1200 ng/mL for bupropion and 2000 ng/mL for olanzapine and trazodone. Figures 5.1-5.24 show the linearity graphs for all 23 drugs studied. Correlation coefficients (r^2) were greater than 0.98 for all drugs except chlorpromazine, fluoxetine, desimprarnine, and norvenlafaxine. It was found that the LODs, LOQs, and percent recoveries varied for each particular drug. The LOQs ranged from 30 ng/mL (clozapine) to 1200 ng/mL (hydroxyzine). The LODs ranged from 10 ng/mL (clozapine) to 390 (hydroxyzine). Percent recoveries ranged from 105.1% (loxapine) to 10.6% (norsertraline). (Table 2)

Precision data was generated for the six drugs at two concentrations: 1000 ng/mL and 250 ng/mL. For within day precision at 250 ng/mL, the CVs ranged from 3.3% to 10.8%. At 1000 ng/mL, the CVs ranged from 0.2% to 7.4%. For the between day precision at 250 ng/mL the CVs ranged from 3.8% to 24.3% and from 5.7% to 11.7% at 1000ng/mL. The accuracy of the assay for each of the six drugs at 250 ng/mL was greater than 92.0%. (Table 3)

Drug	Linearity (r^2)	LOQ (ng/mL)	LOD (ng/mL)	% Recovery
Amitriptyline	0.998	50	25	45.7
Nortriptyline	0.999	50	25	43.0
Amoxapine	0.983	140	40	82.1
Bupropion*	0.999	340	100	37.7
Chlorpromazine	0.943	460	140	43.9
Citalopram	0.999	50	20	66.9
Clozapine	0.999	30	10	80.1
Doxepin	0.992	130	40	84.3
Nordoxepin	0.994	260	80	68.5
Fluoxetine	0.891	600	200	15.9
Haloperidol	0.985	450	150	17.2
Hydroxyzine	0.996	1200	390	10.8
Imipramine	0.995	170	50	83.3
Desipramine	0.973	290	90	64.4
Loxapine	0.995	50	10	105.1
Mirtazapine	0.999	30	10	73.3
Olanzapine**	0.991	210	60	42.0
Sertraline	0.997	480	140	19.4
Norsertraline	0.985	600	200	10.6
Thioridazine	0.996	300	90	20.6
Trazodone**	0.999	870	260	97.5
Venlafaxine	0.999	50	20	78.0
Novenlafaxine	0.867	950	284	67.5

Table 2: Assay Validation Results

*** Limit of linearity 1200 ng/mL, **Limit of linearity 2000 ng/mL**

Figure 5.1: Amitiptyline

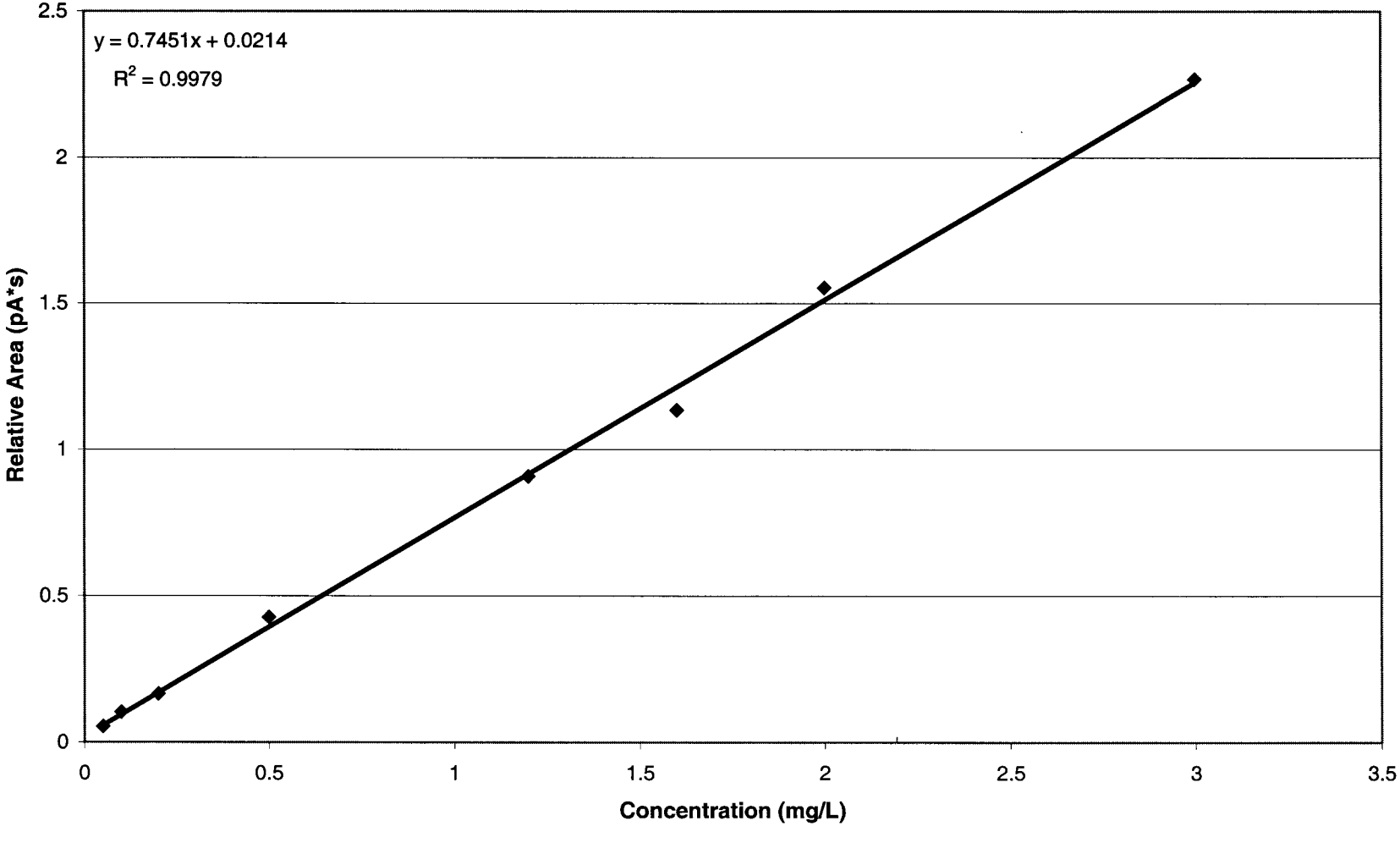


Figure 5.2: Nortriptyline

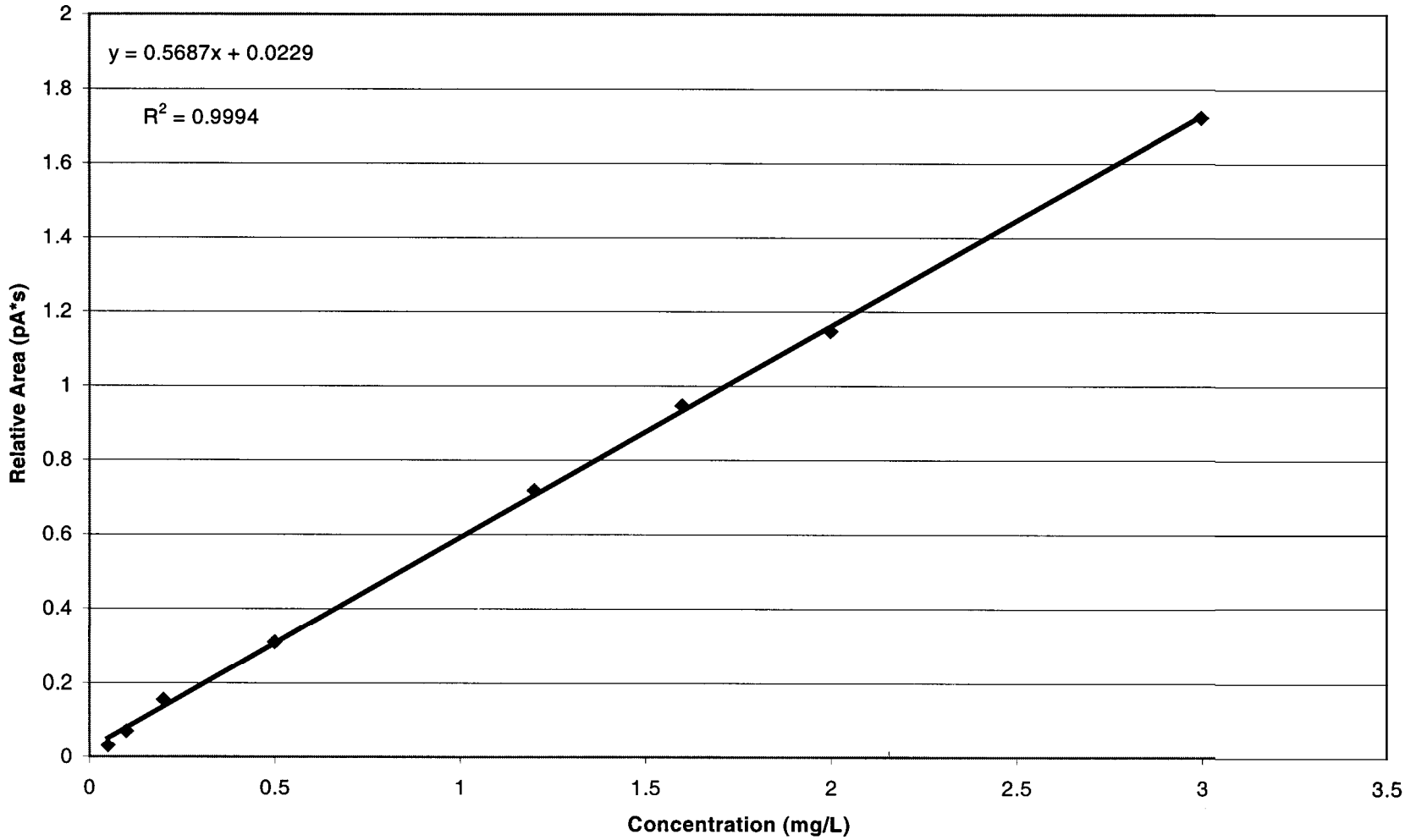


Figure 5.3: Amoxapine

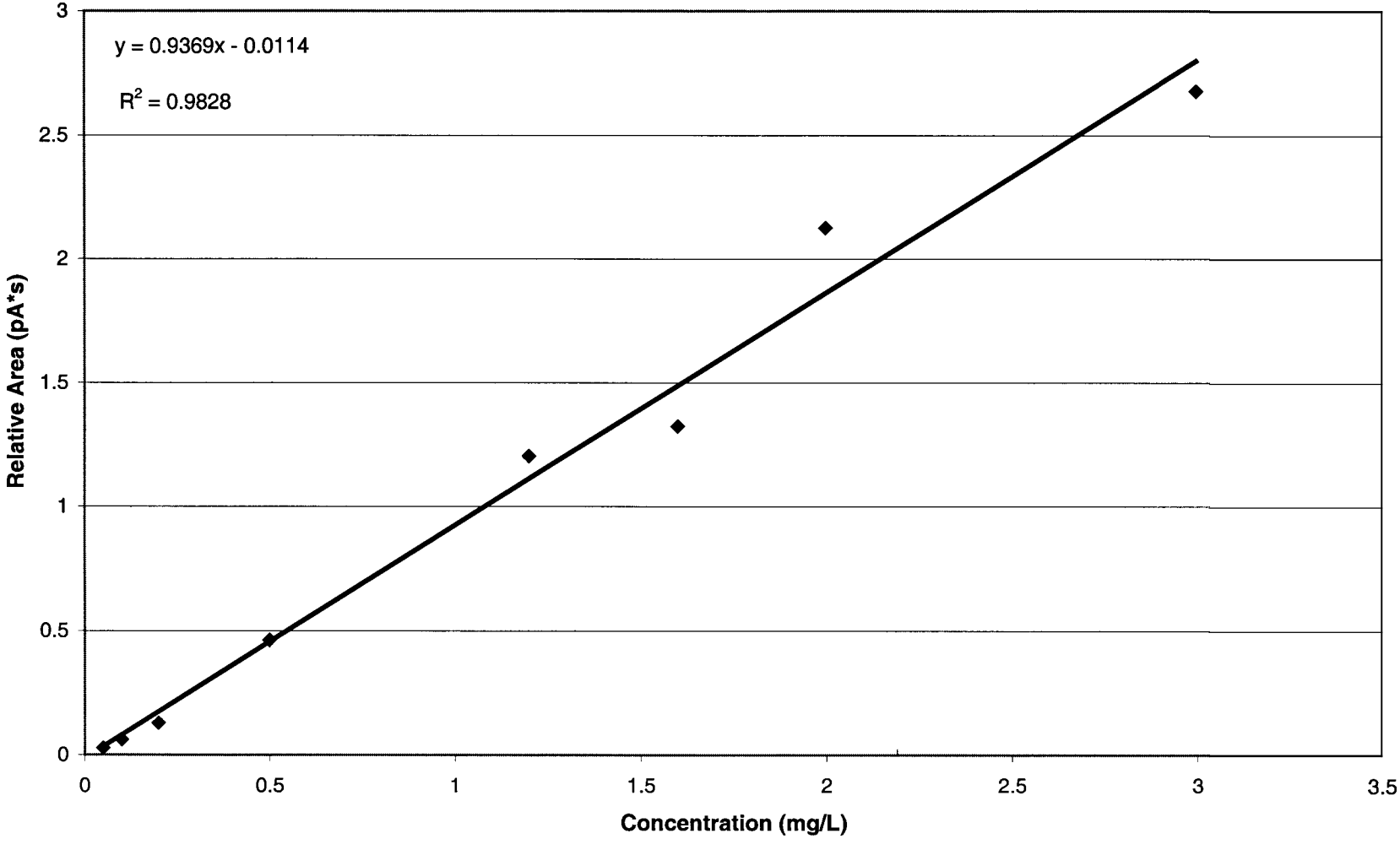


Figure 5.4: Bupropion

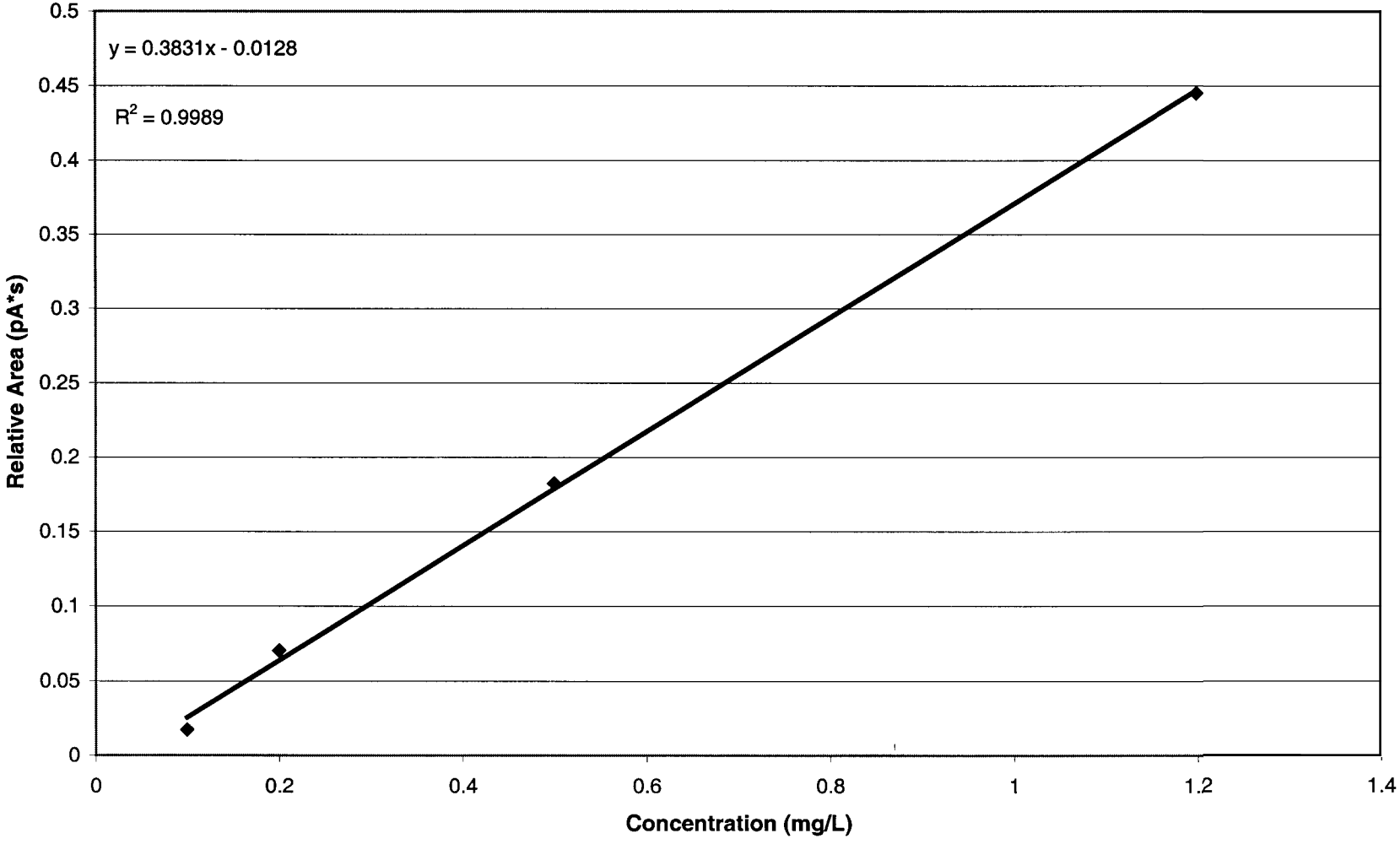


Figure 5.5: Chlorpromazine

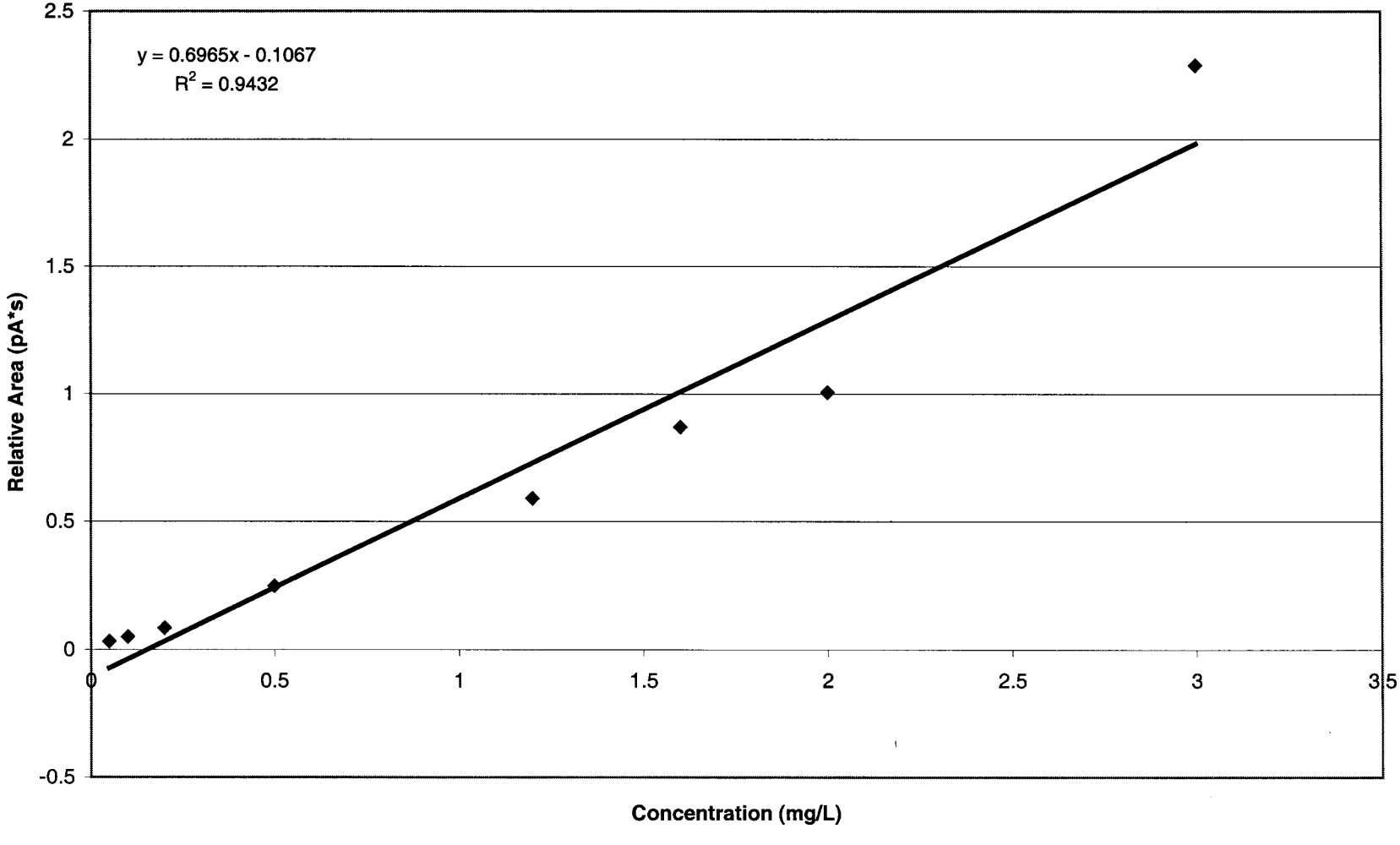


Figure 5.6: Citalopram

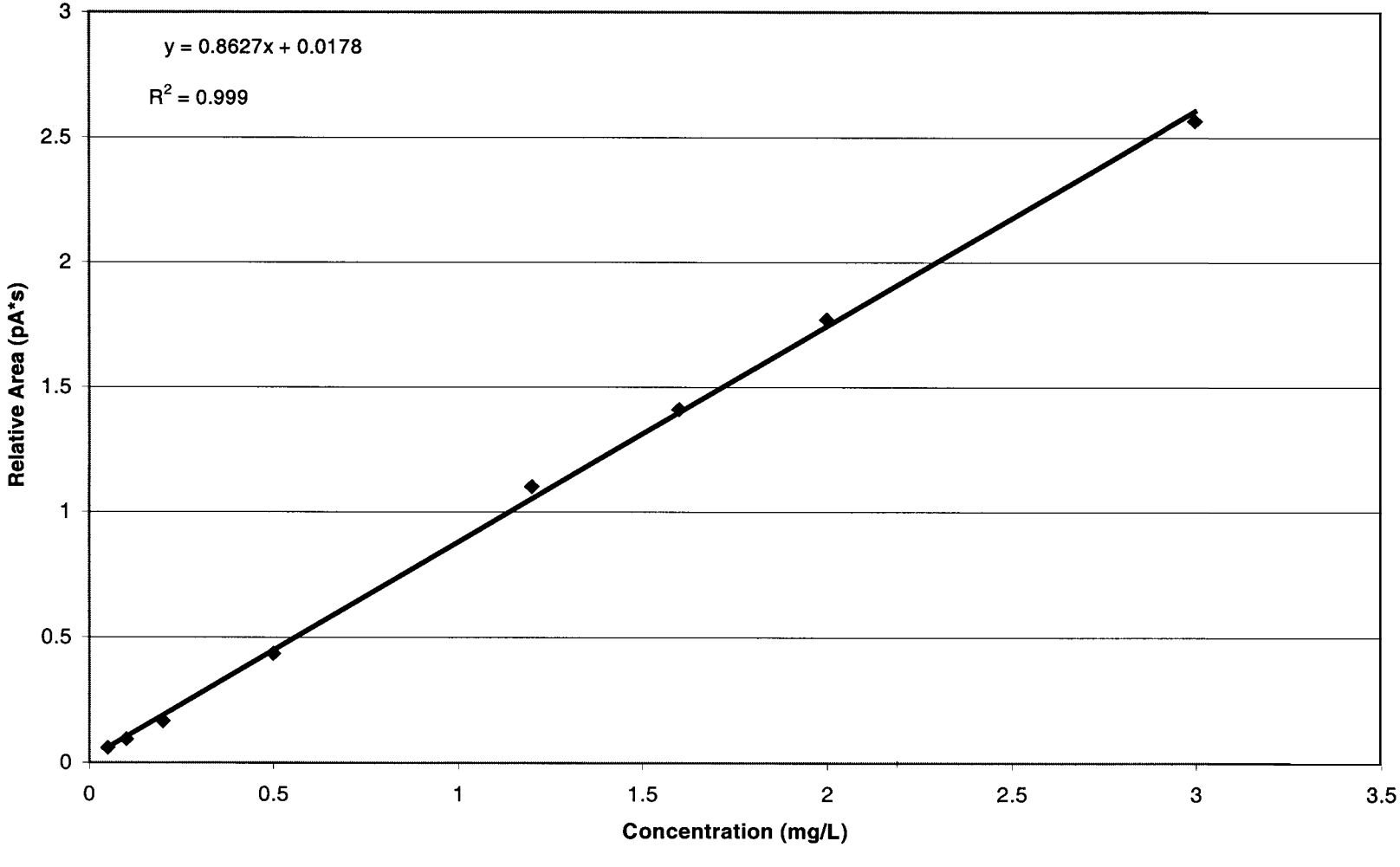


Figure 5.7: Clozapine

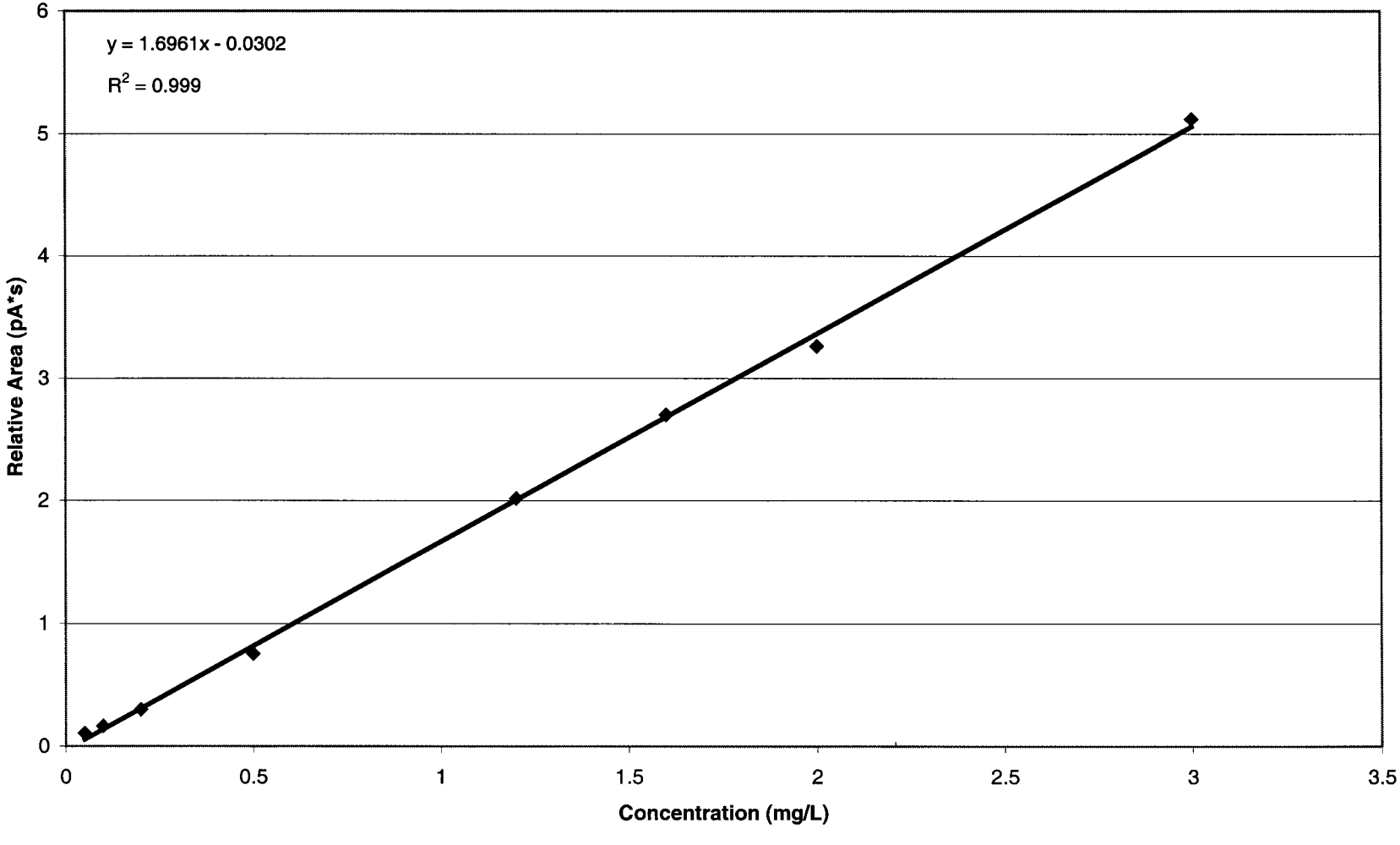


Figure 5.8: Doxepin

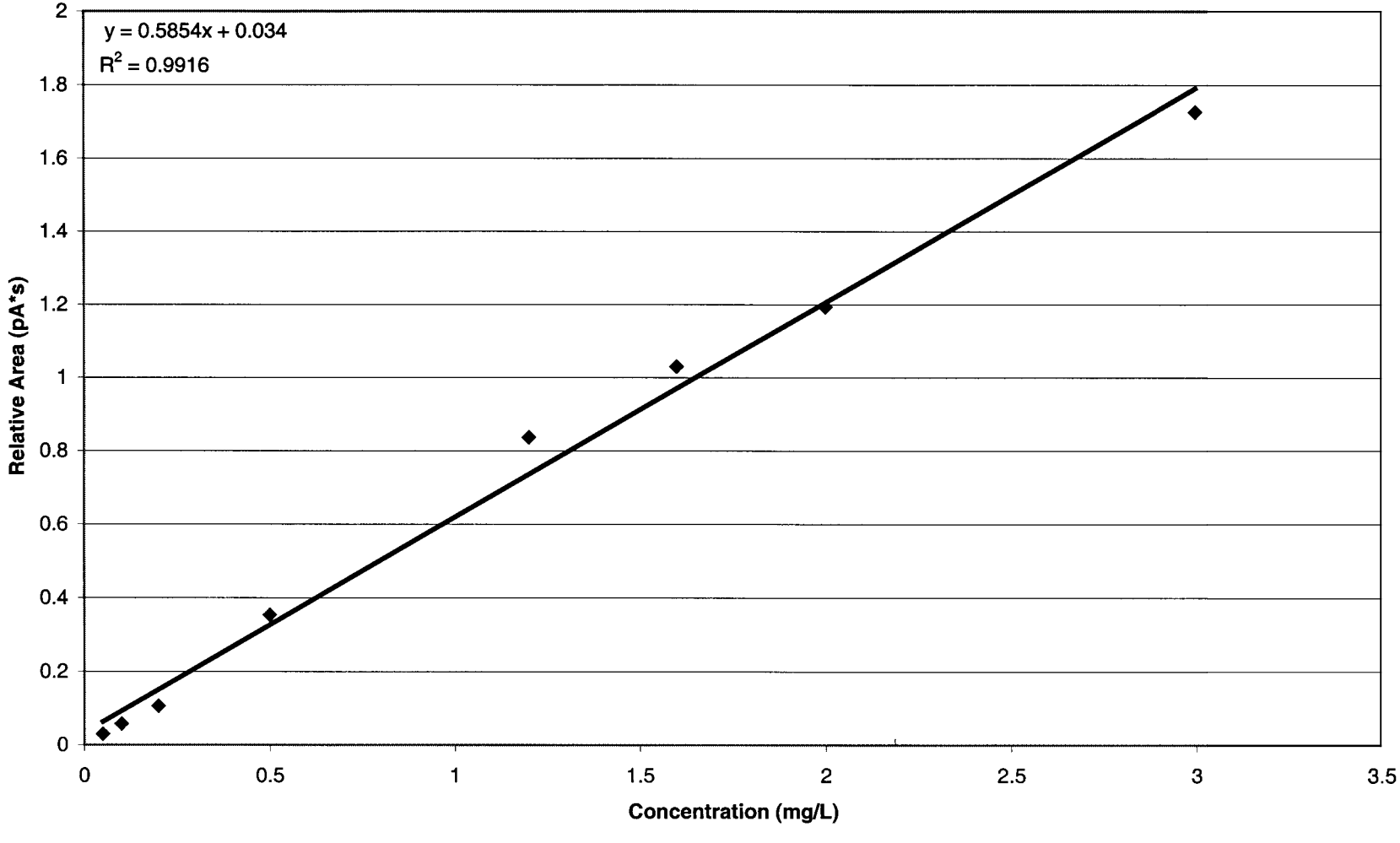


Figure 5.9: Nordoxepin

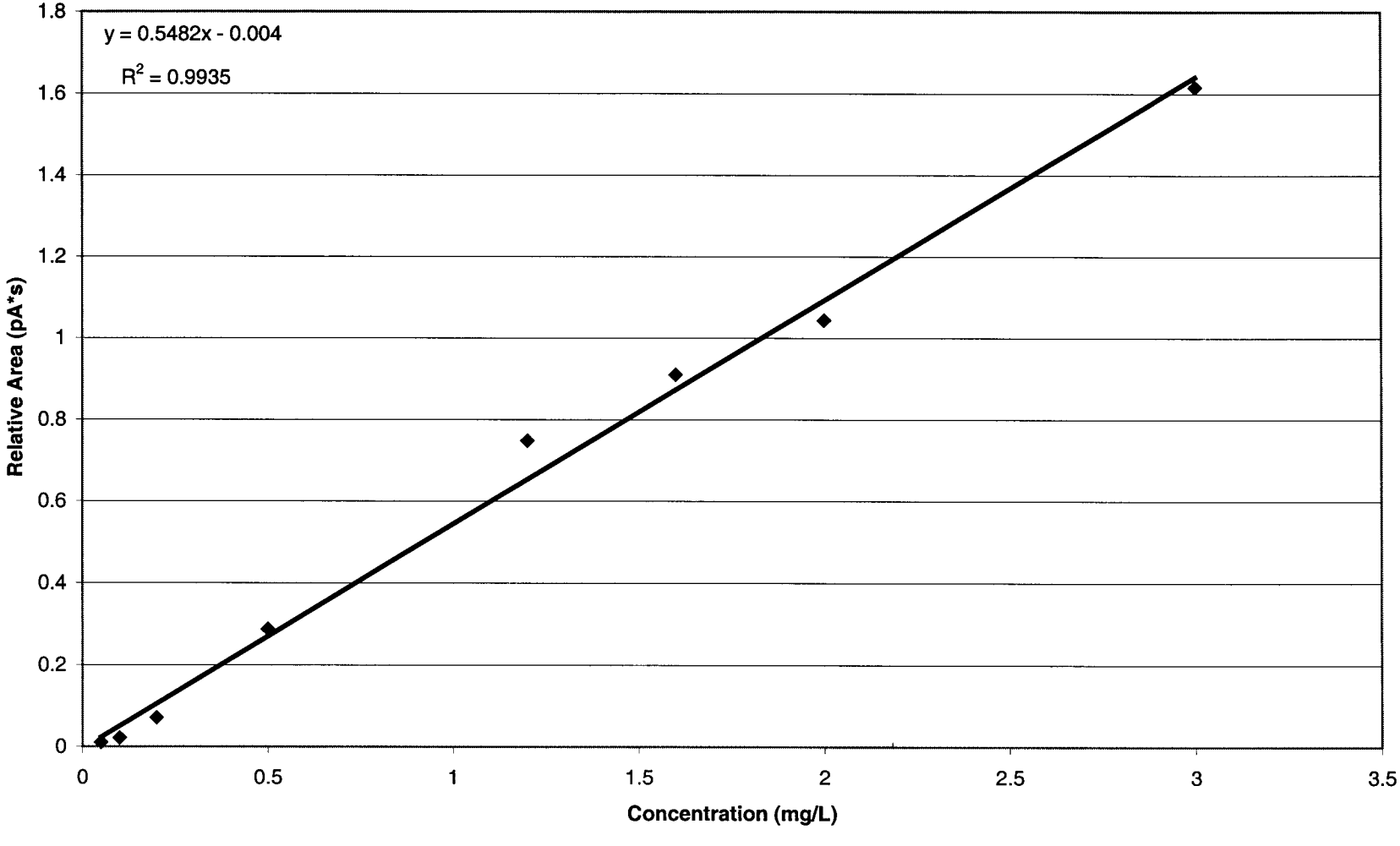


Figure 5.10: Fluoxetine

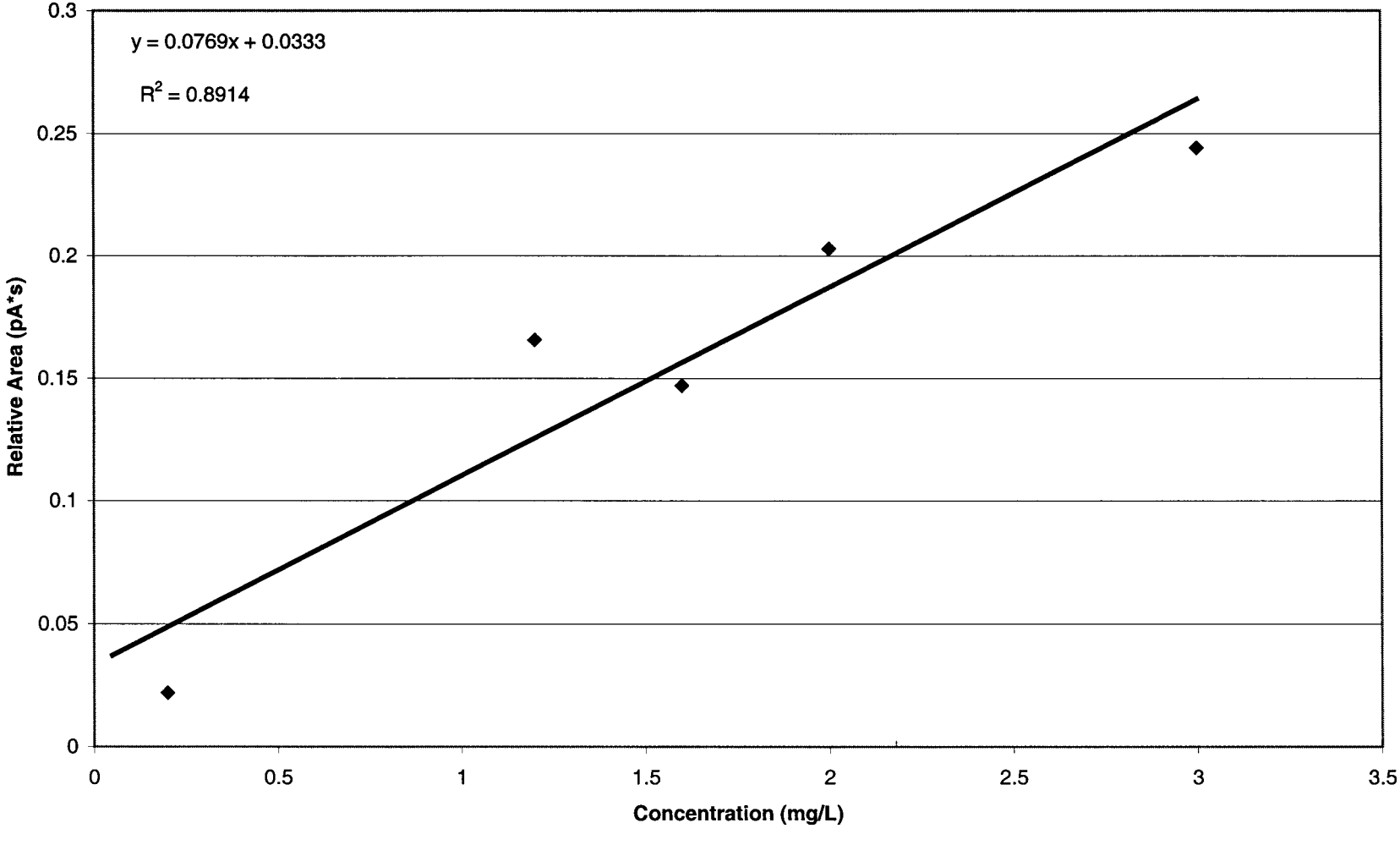


Figure 5.11: Haloperidol

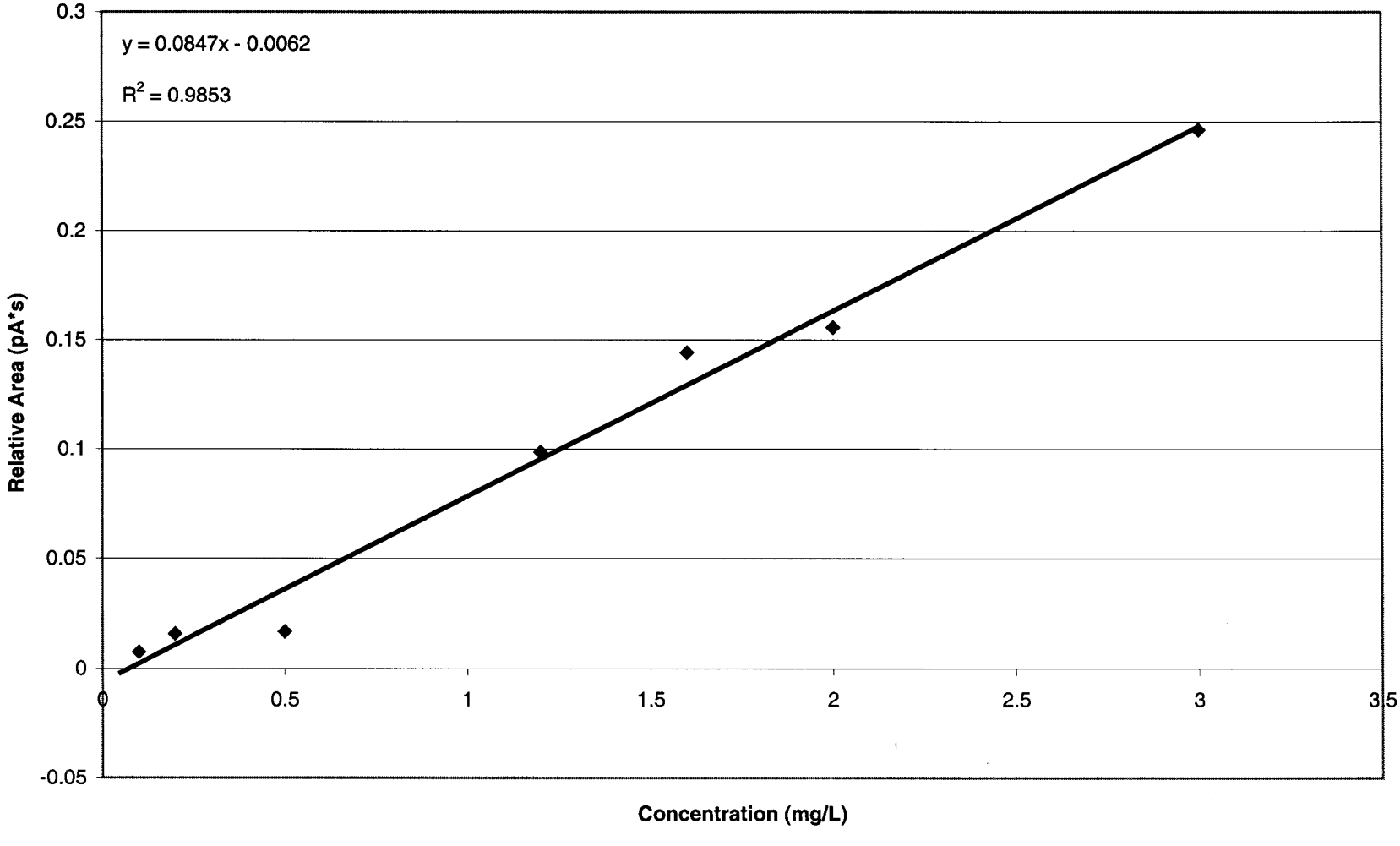


Figure 5.12: Hydroxyzine

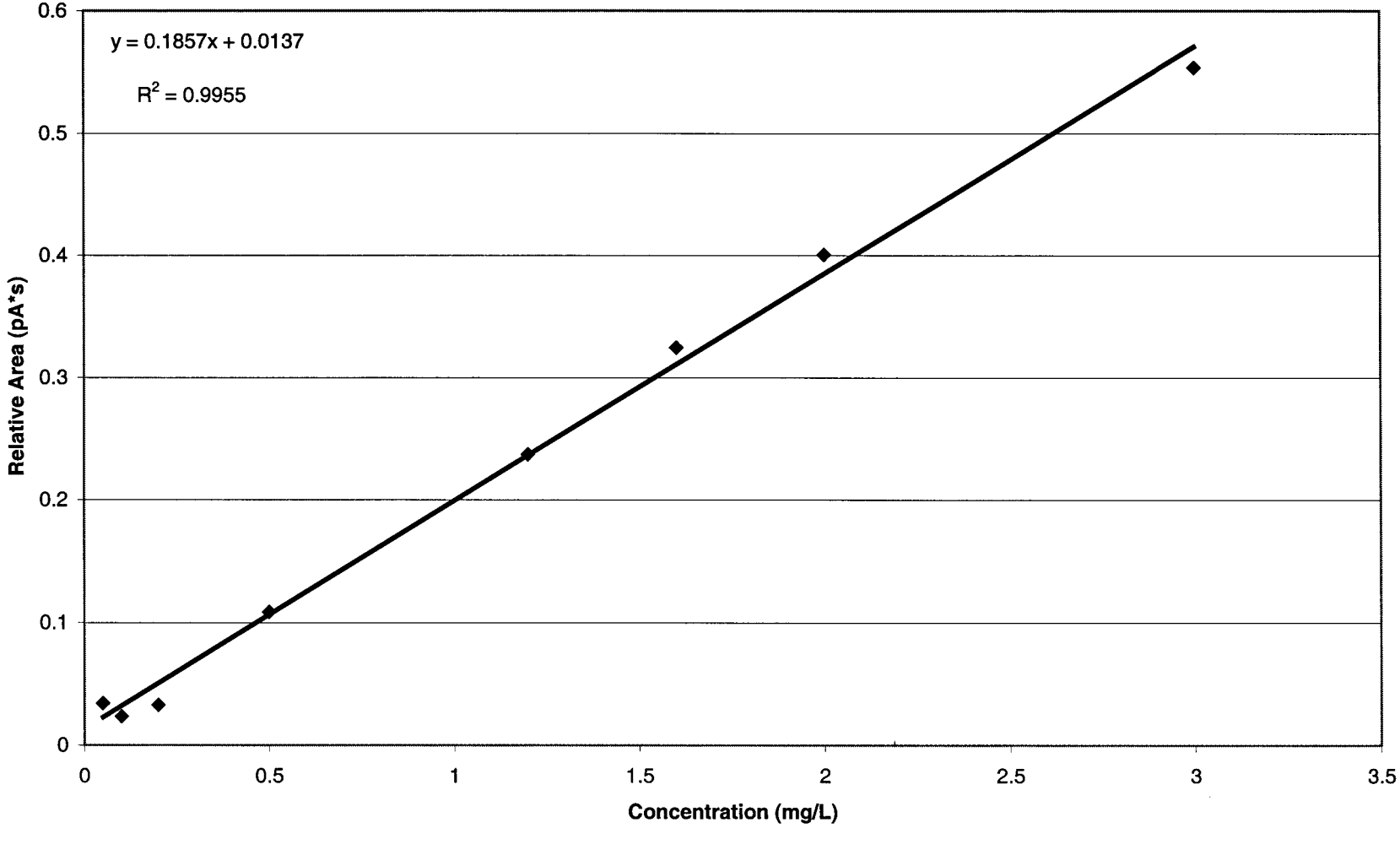


Figure 5.13: Imipramine

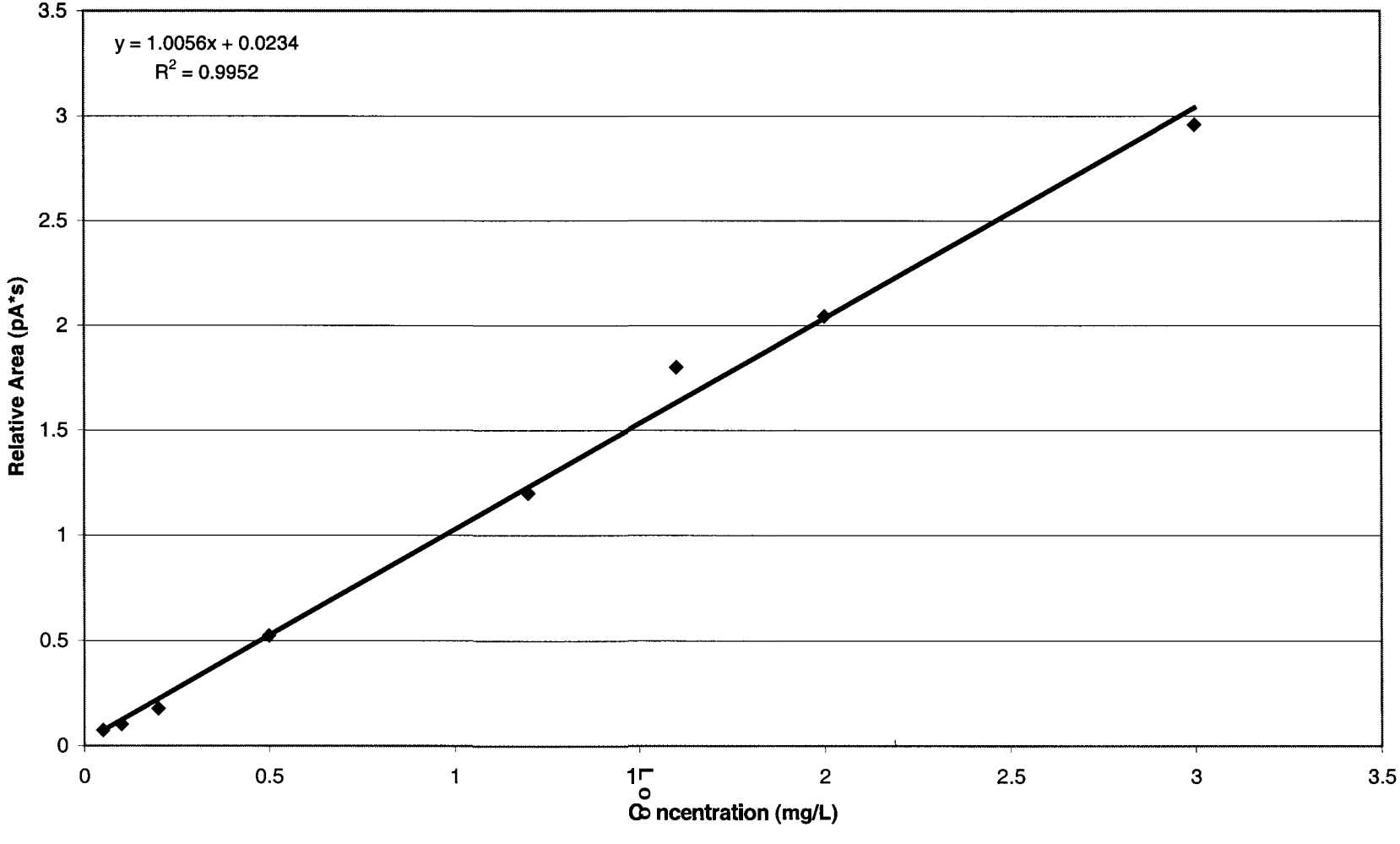


Figure 5.14: Desipramine

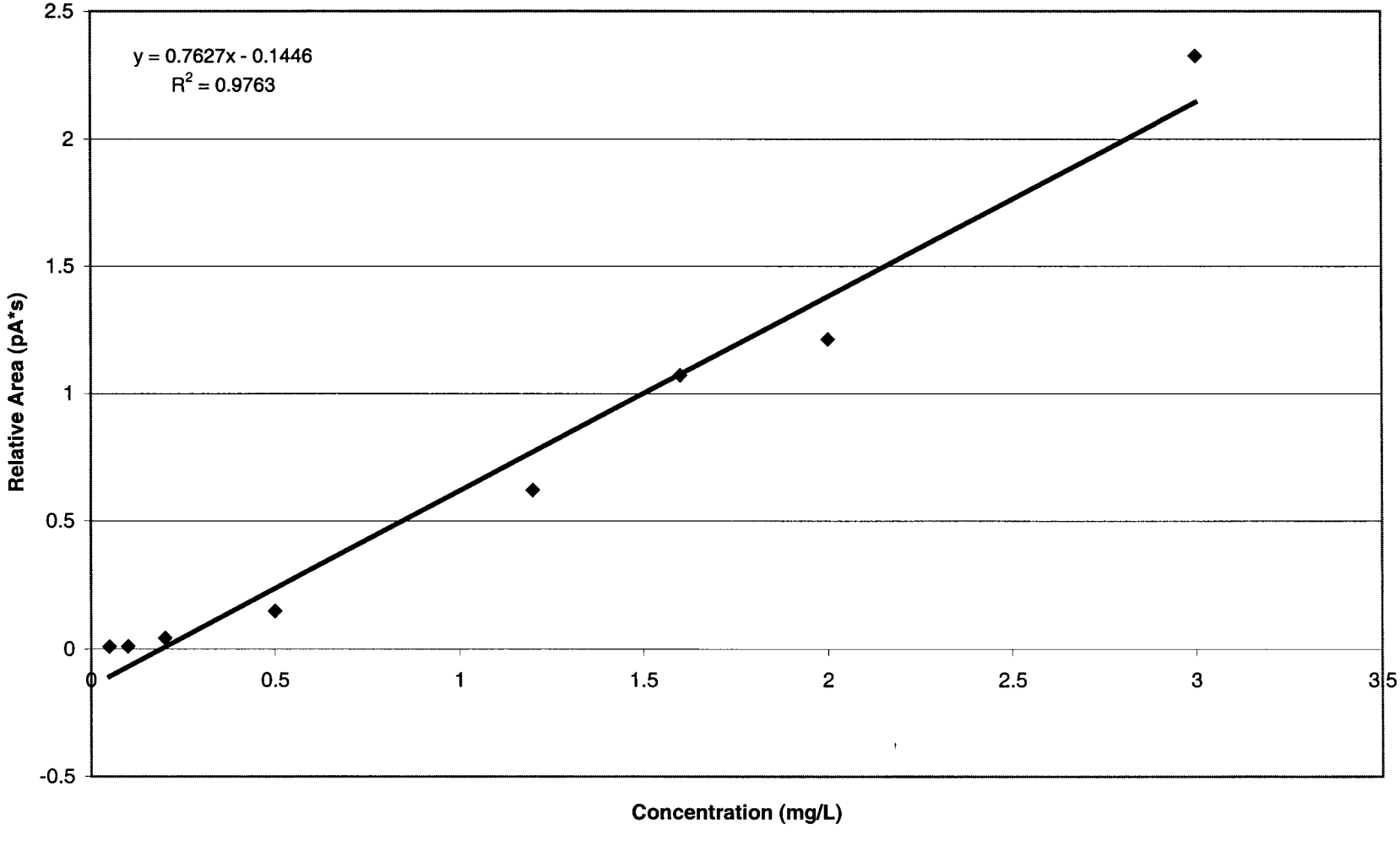


Figure 5.15: Loxapine

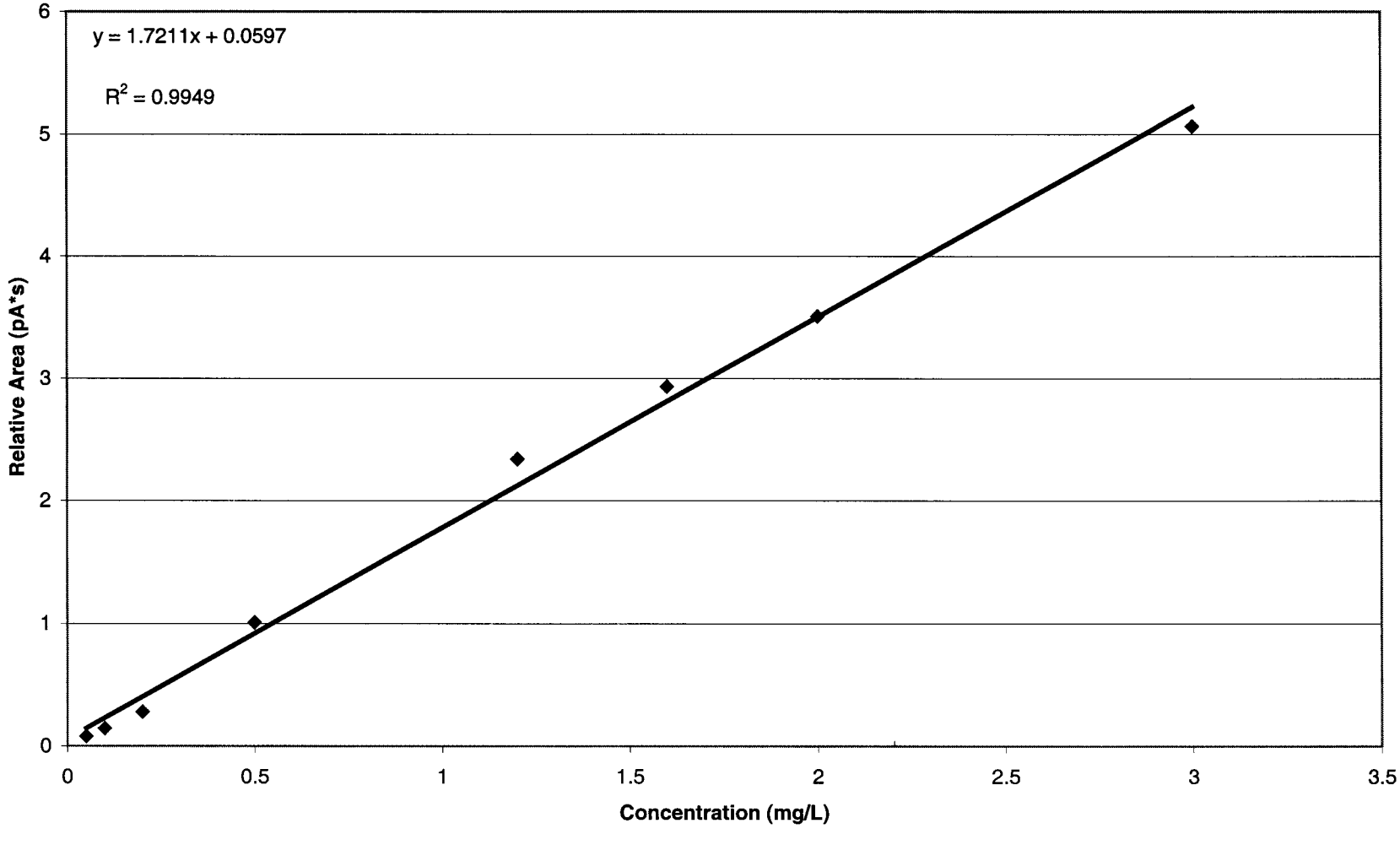
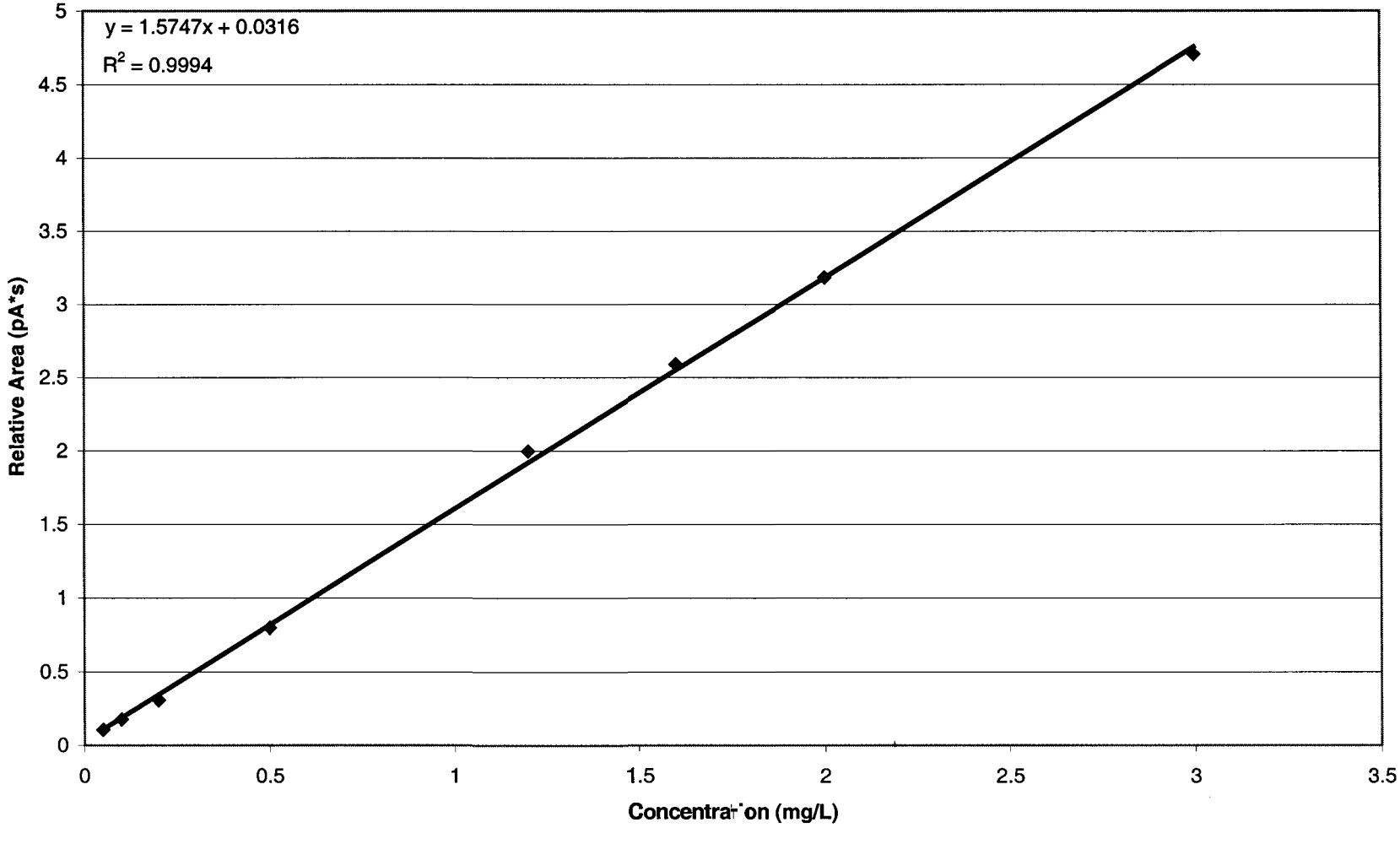


Figure 5.16: Mitrazapine



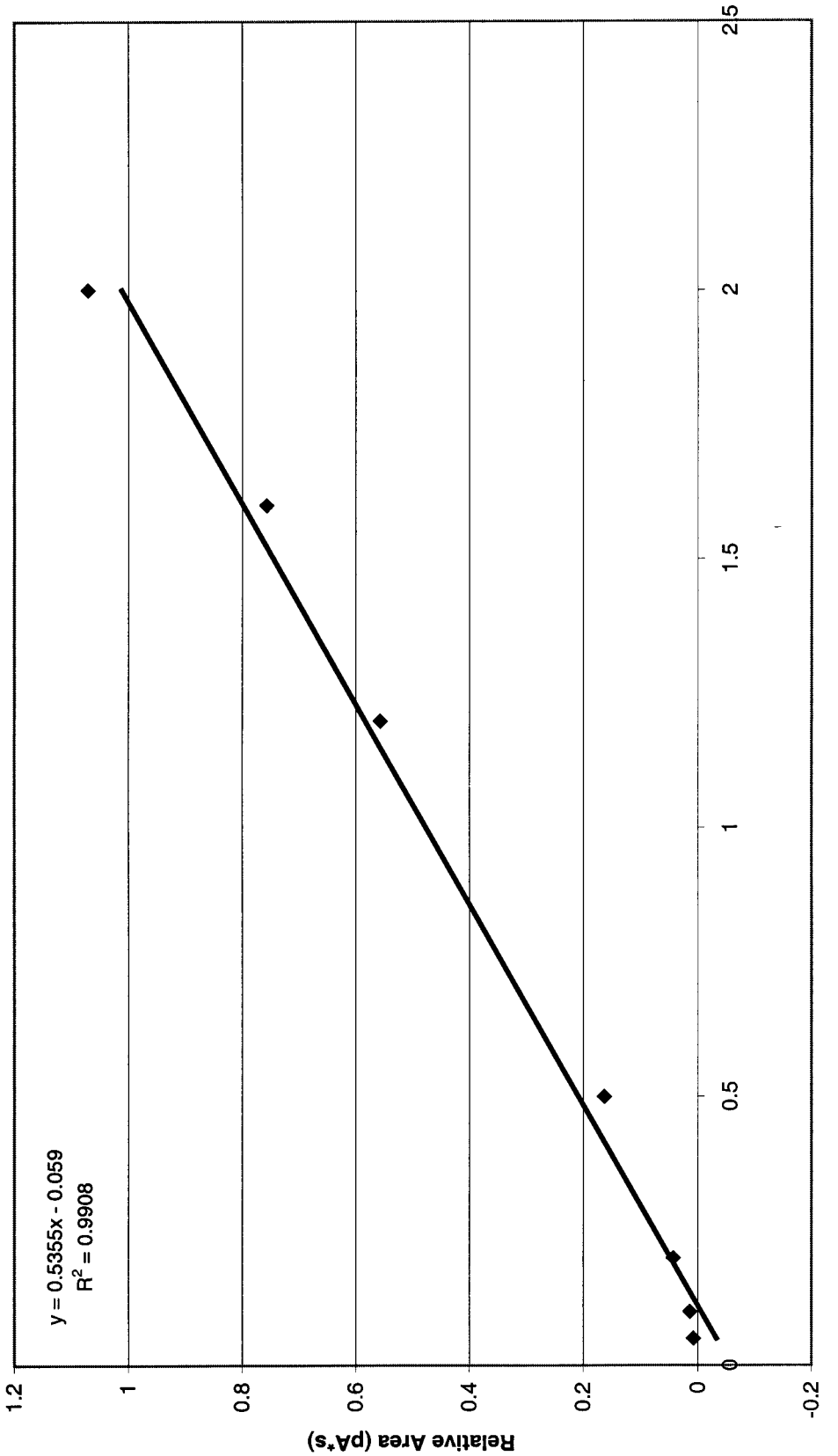
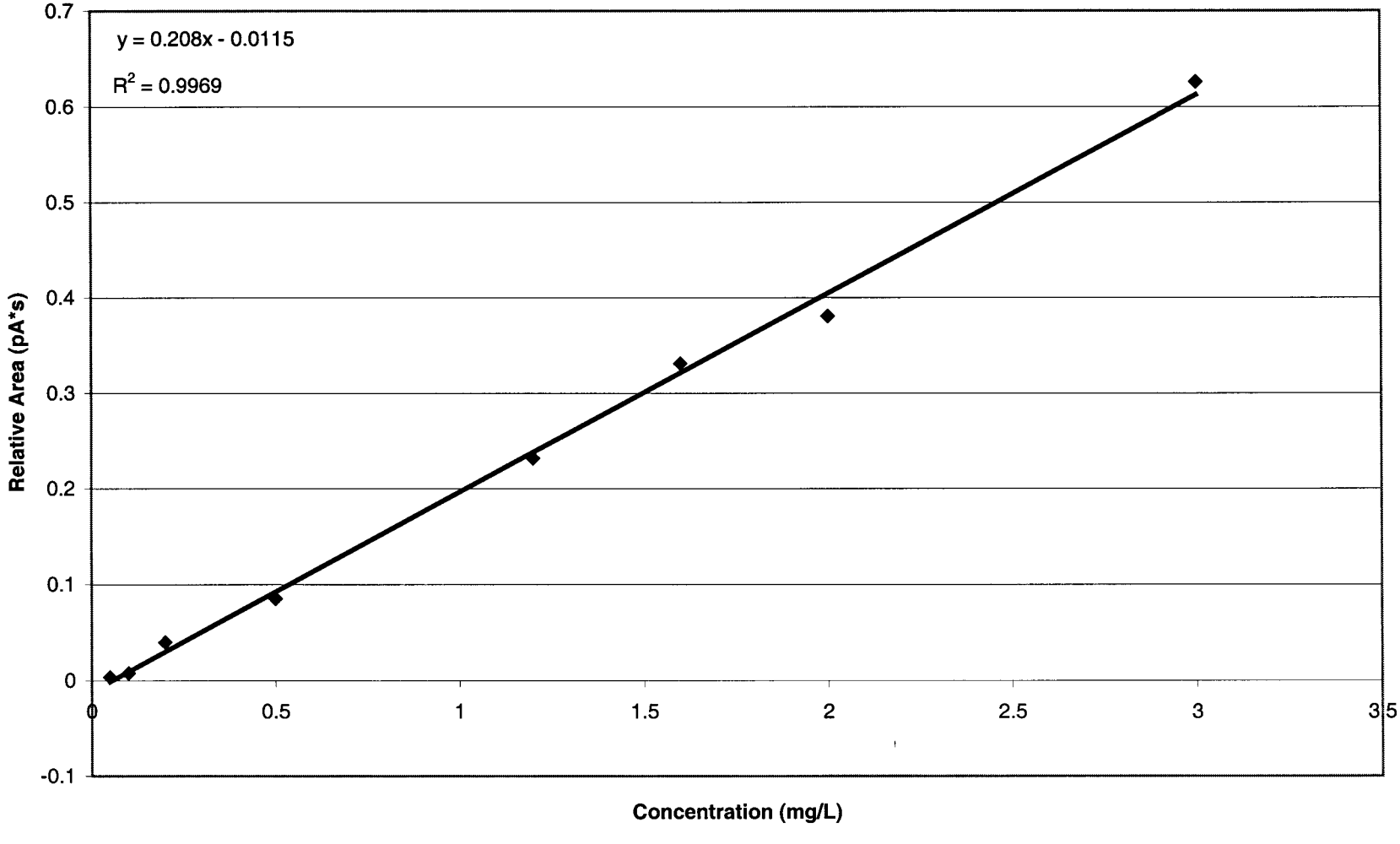


Figure 5.17: Olanzapine

Figure 5.18: Sertraline



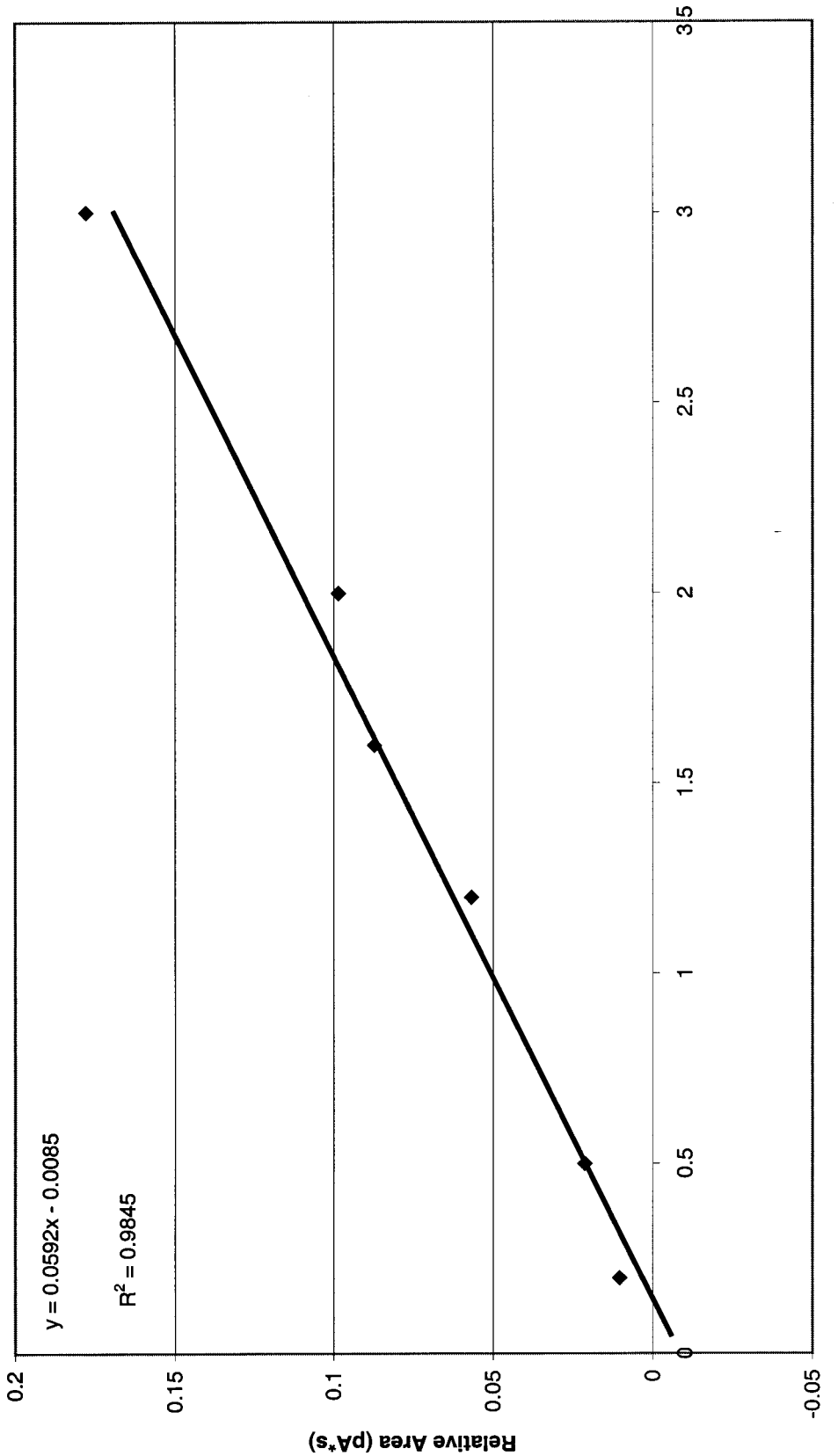


Figure 5.19: Norsertraline

Figure 5.20: Thioridazine

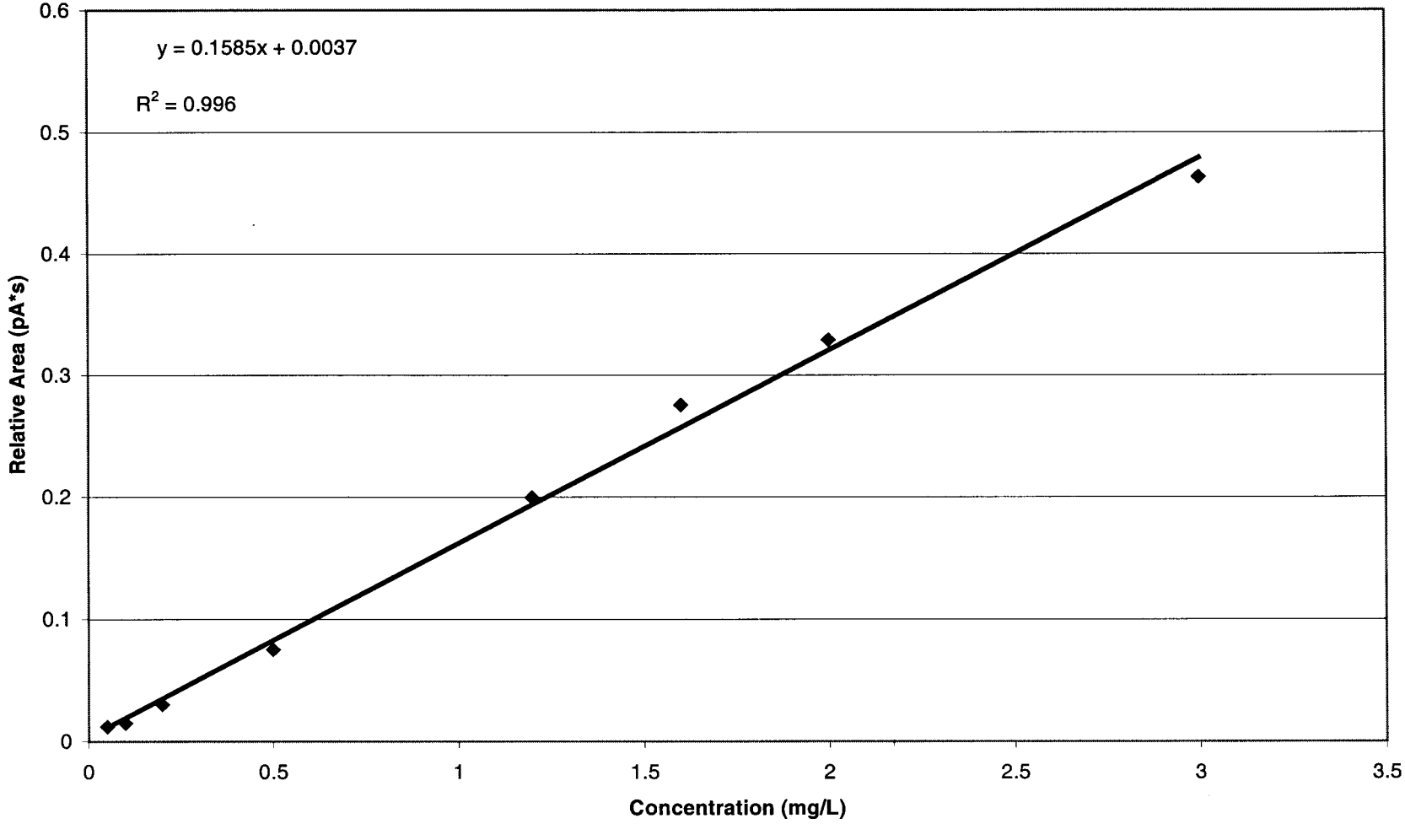


Figure 5.21: Trazodone

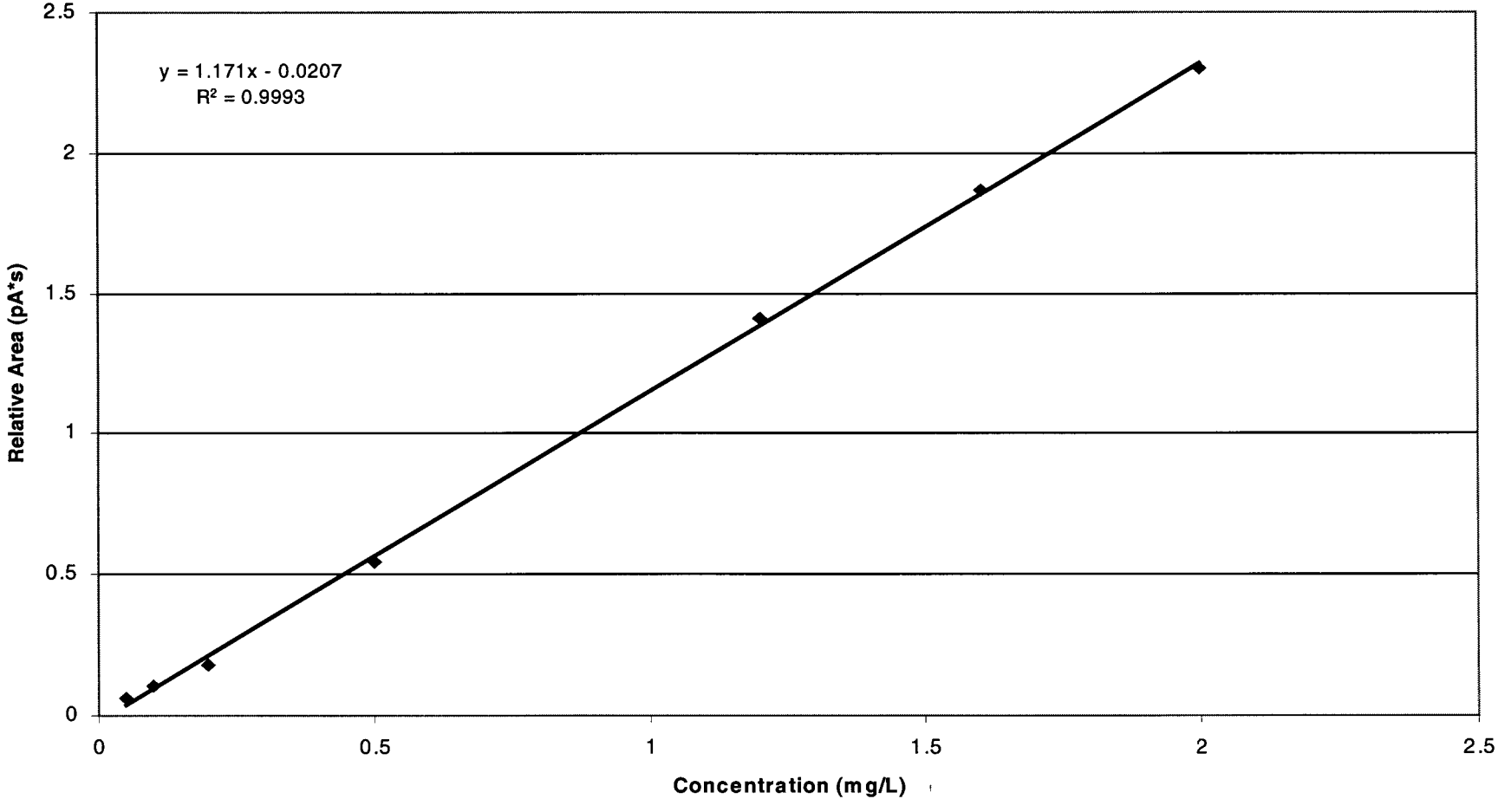


Figure 5.22: Venlafaxine

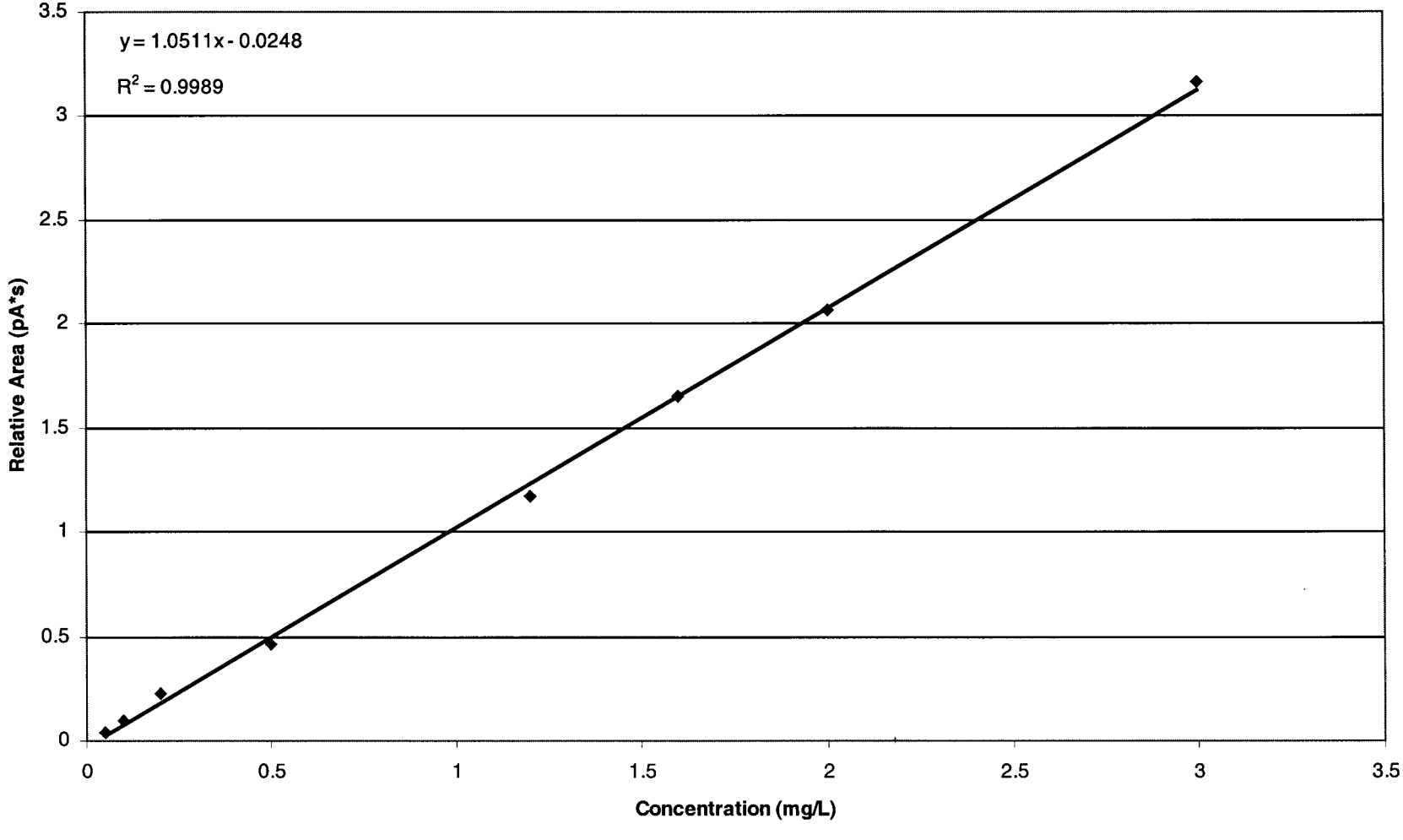
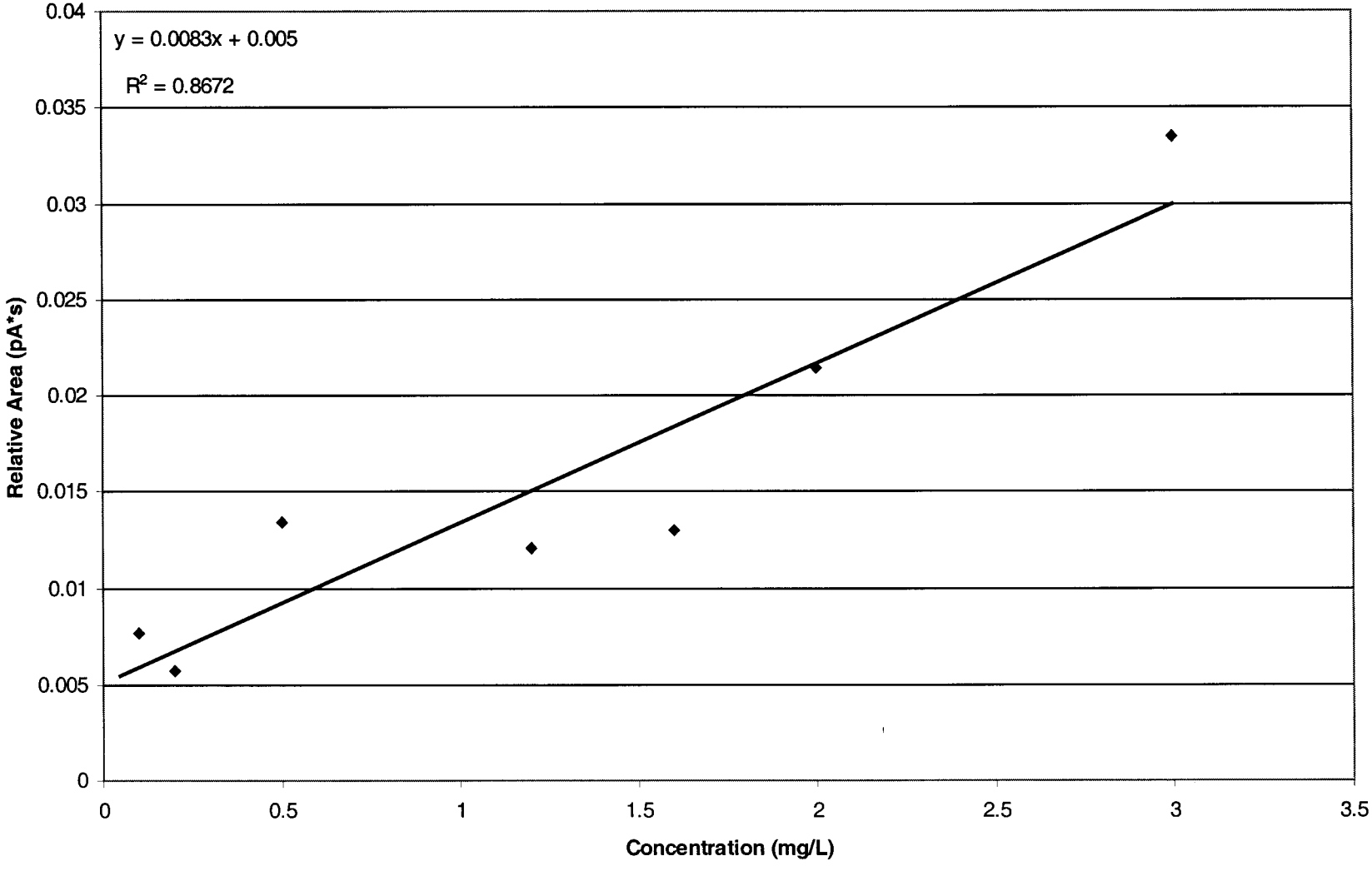


Figure 5.23: Norvenlafaxine



Drug	%CV (within day) (N=9)		%CV (between day) (N=5)		Accuracy (%)
	250 ng/mL	1000 ng/mL	250 ng/mL	1000 ng/mL	250 ng/mL
Fluvoxamine	10.77	2.61	24.29	10.3	92.6
Amitriptyline	3.05	1.83	7.90	8.12	99.0
Sertraline	7.14	0.18	12.18	5.72	95.3
Chlorpromazine	3.26	0.77	4.80	6.13	96.9
Loxapine	4.92	1.44	3.80	6.88	95.6
Thioridazine	9.14	7.45	12.70	11.68	96.5

Table 3: Precision and Accuracy Results

Case Results

Qualitative and quantitative analysis were conducted by comparison with drugs standards of known concentration that contained an internal standard. Qualitative analysis was conducted by comparing the retention time of the suspected drug(s) in the samples to the retention time of known drugs in the standards. Quantitative analysis was achieved by comparing the ratios of the drug peak area in the sample to the internal standard peak area in the sample versus the corresponding drug peak area in the standard to the internal standard peak area. All results were reported as ng/g.

Of the 24 cases that were controls, 13 were negative; no drugs were found by blood or brain analysis. Eight other controls were positive for drugs other than antidepressants and antipsychotics in the blood and no new findings were revealed by brain analysis. In the Iast three controls, drugs other than those found in the blood were discovered by brain analysis, however, these drugs were not antidepressants or antipsychotics and no further analysis was performed. (Table 4)

Of the remaining 37 cases, 16 were negative, no drugs of any type were detected in the blood or brain analysis. Seven of the cases were positive for drugs other than antidepressants and antipsychotics in the blood and no new findings were revealed by brain analysis. In ten cases, antidepressant and/or antipsychotic drugs were found by blood analysis and then verified by brain analysis. In the remaining four cases, brain analysis found antidepressant and/or antipsychotic drugs that were undetected by blood analysis. These results were confirmed by GC/MS. (Table 4) One example is case 22. In this case, no drugs of any type were detected by blood analysis. On the other hand, brain analysis indicated the presence of sertraline and its metabolite, norsertraline, in both the

frontal cortex (1350 ng/mL and 3940 ng/mL respectively) and the cerebellum (1180 ng/mL and 3510 ng/mL respectively). (Figures 6.1 and 6.2)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (nglg)	GC Cerebellum (ng/g)
1*	Negative	Negative	Negative
2*	Negative	Negative	Negative
3	**Negative**	**Sertraline (140)** **Norsertraline (4100)**	**Sertraline (97)**
4*	Negative	Negative	Negative
5*	Negative	Negative	Negative
6*	Lidocaine (6000)	Lidocaine (1640)	Lidocaine (1970)
7	Negative	Negative	Negative
8*	Negative	Negative	Negative
9*	Propoxyphene (130)	Propoxyphene (30)	Propoxyphene (32)
10*	Negative	Negative	Negative
11*	Negative	Negative	Negative
12*	Negative	Dextromethorphan (200) Doxylamine (66)	Dextromethorphan (253) Doxylamine (80)

Table 4: Result Comparison of Brain Tissue Analysis (*=control)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (ng/g)	GC Cerebellum (nglg)
13	Negative	Negative	Negative
14*	Negative	Negative	Negative
15	Cocaine (300)	Cocaine (284)	Cocaine (208)
16	Diazepam (370) Desmethyldiazepam (120)	Diazepam (277) Desmethyldiazepam (276)	Diazepam (1030)
17*	Negative	Negative	Negative
18	**Bupropion**	**Positive**	**Positive**
19*	Negative	Negative	Negative
20	**Sertraline (190)**	**Sertraline (1360)** **Norsertraline (2000)**	**Sertraline (737)** **Norsertraline (1050)**
21	Negative	Negative	Negative
22	**Negative**	**Sertraline (1350)** **Norsertraline (3940)**	**Sertraline (1180)** **Norsertraline (3510)**
23	Lidocaine (1000)	Lidocaine (114)	Lidocaine (126)

Table 4 cont.: Result Comparison of Brain Tissue Analysis (*=control)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (ng/g)	GC Cerebellum (ng/g)
24	Cocaine (570)	Negative	Negative
25	Cocaine (290)	Cocaine (519)	Cocaine (394)
26*	Chlorpheniramine (90)	Negative	Negative
27*	Negative	Negative	Negative
28	Lidocaine (560)	Negative	Negative
29	**Sertraline (330) Norsertraline Positive**	**Tissue not available**	**Sertraline (410) Norsertraline (386)**
30*	Negative	Meperidine (236)	Meperidine (183)
31	Negative	Negative	Negative
32	**Chlorpromazine (1950)** Dextromethorphan (350)	**Chlorpromazine (1240)** Dextromethorphan (242)	**Chlorpromazine (698)** Dextromethorphan (155)
33	**Fluoxetine (1720)**	**Fluoxetine (2160)**	**Fluoxetine (2560)**

Table 4 cont.: Result Comparison of Brain Tissue Analysis (*=control)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (ng/g)	GC Cerebellum (ng/g)
34*	Negative	Negative	Negative
35*	Diltiazem (180)	Diltiazem (127)	Diltiazem (93)
36	Negative	Negative	Negative
37*	Negative	Negative	Negative
38	Negative	Negative	Negative
39*	Negative	Negative	Negative
40	**Negative**	**Sertraline (1060)** **Norsertraline (2020)**	**Sertraline (726)** **Norsertraline (1350)**
41	Negative	Negative	Negative
42	Negative	Negative	Negative
43*	Lidocaine (8534)	Lidocaine (7230)	Lidocaine (11530)
44	Meperidine (259) **Sertraline (228)** **Norsertraline (545)**	Meperidine (108) **Sertraline (1150)** **Norsertraline (2850)**	Meperidine (127) **Sertraline (1070)** **Norsertraline (2650)**

Table 4 cont.: Result Comparison of Brain Tissue Analysis (*=control)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (ng/g)	GC Cerebellum (ng/g)
45	Lidocaine (1080)	Negative	Negative
46	Negative	Chlorpheniramine (9.4)	Tissue not available
47	Negative	Negative	Negative
48*	Negative	Chlorpheniramine (61)	Chlorpheniramine (43)
49	Diphenhydramine (110)	Negative	Negative
50	**Trazodone (180)**	**Trazodone (45)**	**Negative**
51	Venlafaxine (2690)	Venlafaxine (1580) **Fluoxetine (1440)**	Venlafaxine (1470) **Fluoxetine (650)**
52	Negative	Negative	Negative
53	**Olanzapine (2580)**	**Olanzapine (240)**	**Negative**
54	Lidocaine (3130)	Negative	Negative
55	Negative	Negative	Negative
56*	Lidocaine (4690)	Lidocaine (3910)	Lidocaine (5200)

Table 4 cont.: Result Comparison of Brain Tissue Analysis (*=control)

Case #	Heart Blood (ng/mL)	GC Frontal Cortex (ng/g)	GC Cerebellum (ng/g)
57	**Clomipramine (120)** **Fluoxetine (200)** Oxycodone (Positive)	**Not tested for** **Fluoxetine (1750)**	**Not tested for** **Fluoxetine (960)**
58	Cocaethylene (210) Cocaine (270)	Not tested for Cocaine (141)	Not tested for Cocaine (65)
59	**Amitriptyline (260)** **Nortriptyline (260)**	**Tissue not available**	**Amitriptyline (99)** **Nortriptyline (217)**
60	Negative	Negative	Negative
61	Negative	Negative	Negative

Table 5 cont.: Result Comparison of Brain Tissue Analysis (*=control)

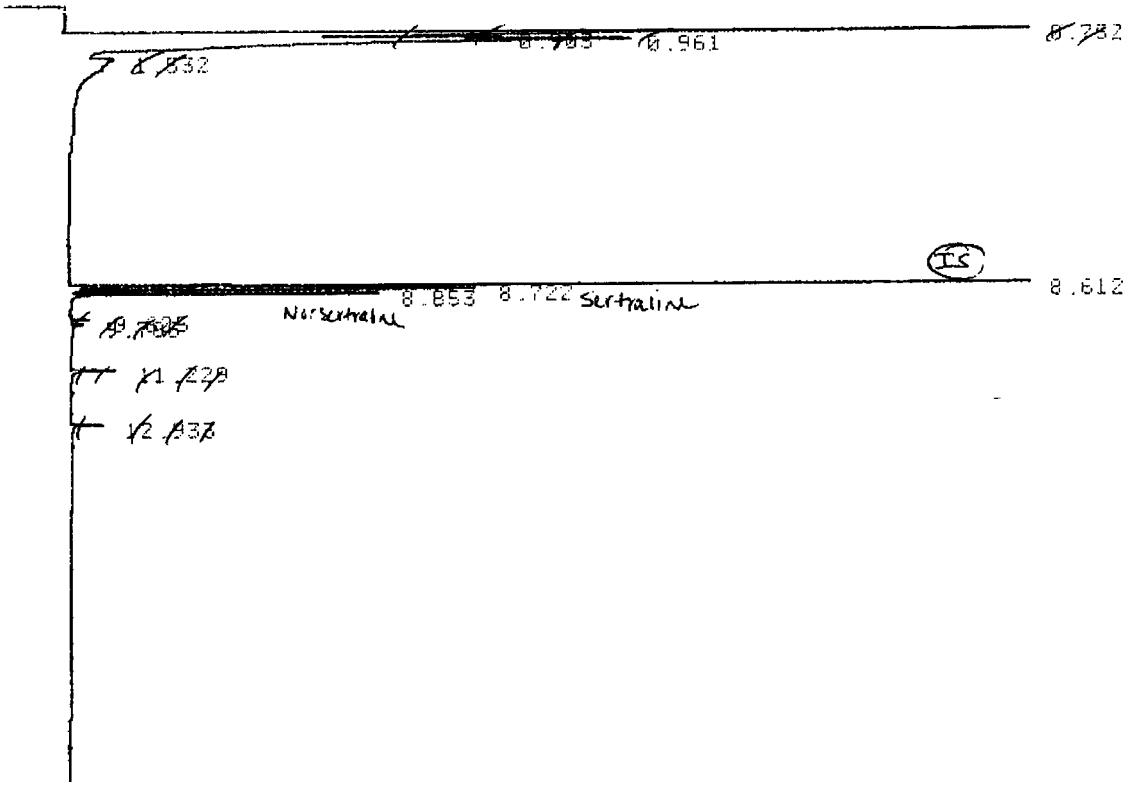


Figure 6.1: Chromatogram of Frontal Cortex from Case 22

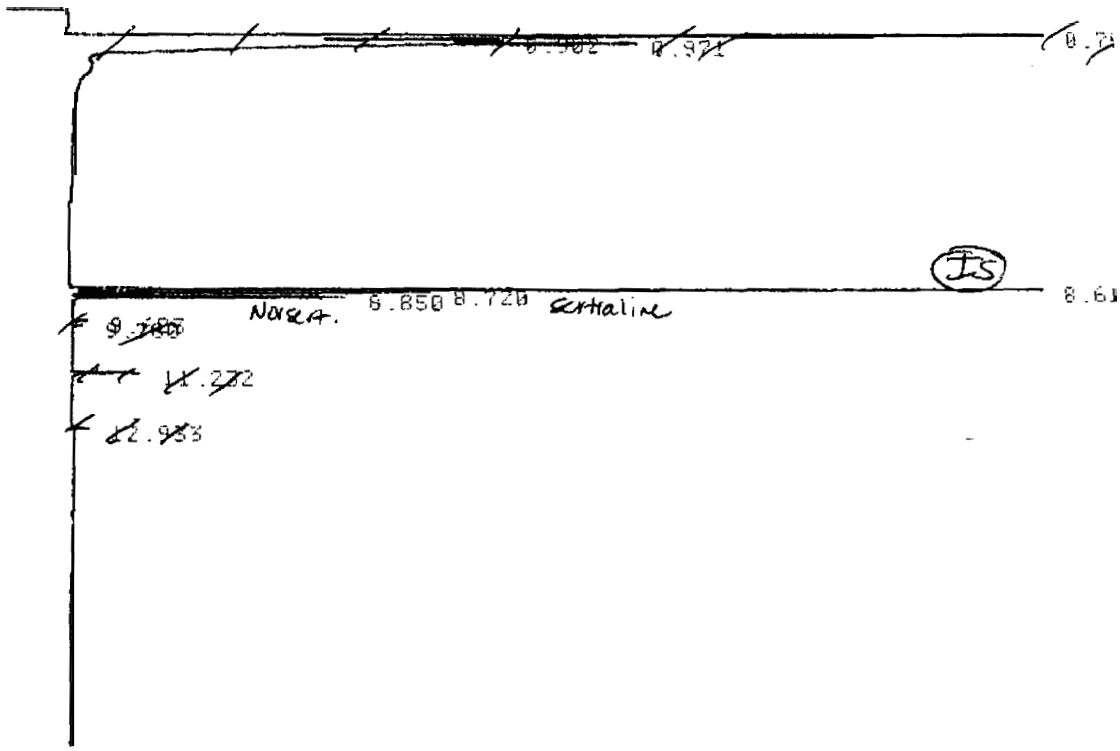


Figure 6.2: Chromatogram of Cerebellum from case 22

Chapter Five

Discussion and Conclusion

In this research, a liquid-liquid extraction procedure was developed for the simultaneous determination of several antidepressant and antipsychotic drugs in human brain tissue. It was found that the LOQ's, LOD's, and percent recoveries varied for each particular drug studied. The method was found to be linear to 3000 ng/mL for all but three of the drugs studied. Trazodone and olanzapine were linear to 2000 ng/mL and bupropion to 1200 ng/mL. Linearity was based on the r^2 value from the linear regression of the line. The majority of r^2 values were greater than 0.990, with values ranging from 0.867 (norvenlafaxine) to 0.999 (multiple drugs). The assay was found to be very accurate and precise with the accuracy of each of the six drugs studied greater than 92%. These results from the validation of the assay developed in this research indicate that it may be a useful method to prepare brain specimens for drug analysis.

The cases analyzed in this research demonstrate the capability of this assay to detect drugs in the brain. When an antidepressant or antipsychotic drug was detected in the blood, it was also detected in the brain in each case. This is very important and indicates that analyzing a brain specimen should not result in an inability to detect these classes of drugs. The concentrations of these drugs differed in the two matrices. Further research should be conducted to explain the differential distribution between matrices. There were a couple of cases where blood analysis detected drugs that were not antidepressants or antipsychotics and brain analysis did not. Since these drugs were not in the two classes researched, no further research was conducted. The four cases where brain analysis was able to detect drugs that were not detected in blood analysis

demonstrate the utility of brain as an alternate testing matrix in death investigations. In three of these cases, sertraline and its metabolite norsertraline were not detected in the blood, but were found in the brain. In some of these cases, the concentrations of the drugs were high and should be expected to be found in the blood, while in other cases, the concentrations were low and their presence may not be expected in the blood. For example, in case #3 blood analysis was unable to detect the presence of any type of drug. Sertraline and norsertraline, however, were found in the frontal cortex at low concentrations and sertraline was found in the cerebellum at a low concentration. Cases **#22** and #40 are examples of higher drug concentrations in the brain and no detectable drug in the blood. In these two cases, blood analysis was unable to detecting the presence of any drug and sertraline and norsertraline were found in both the frontal cortex and cerebellum of the individual. In case #51 blood analysis detected the presence of the antipsychotic drug, venlafaxine. After brain analysis, fluoxetine was detected in both the frontal cortex and cerebellum. These four cases indicate the importance of using alternative matrices. The reason why sertraline and norsertraline were often not detected in blood but were positive in brain analysis is not known. **A** few reasons why this could occur include differential distribution of the drug at death, the time since the drug had been ingested and the half-lives of the drug, which are not known in the brain. Further inquiry into this project could look at this aspect of the research.

The objective of this research was to develop a method capable of detecting and quantitating common antidepressant and antipsychotic drug concentrations in human brain tissue and to indicate the usefulness of brain tissue as a specimen in death investigations. This research has developed method that can simultaneously detect these

drugs in human brain tissue. Furthermore, the results of the case analysis indicate that brain tissue is a useful testing matrix. This research shows that brain tissue analysis can detect drugs that are undetected by blood analysis. Also, if blood is not present at the time of autopsy, brain tissue gives the toxicologist a sample that will yield similar qualitative results in most cases.

The field forensic toxicology is a constantly growing area and new technology and research will continue to improve the field. Further research in this subject could include analyzing more cases to confirm these results. In addition, drugs other than antidepressants and antipsychotics should be studied. The present research was able to detect some of these drugs as well. From this research, the use of brain tissue as an alternative specimen for death investigations appears to be a valuable adjunct to testing in more traditional matrices.

References

(1) Baslet, R.C.; Cravey, R.H. *Introduction to Forensic Toxicology;* Biomedical Publications: Davis, California, 1981.

(2) Curry, A.S. *Poison Detection in Human Organs;* Bannerstone House: Springfield, Illinois, 1963.

(3) Apple, F.S.; Bandt C.M. Liver and Blood Postmortem Tricyclic Antidepressant Concentrations. *Am. J. Clin. Pathol.* **1988,** *89,* 794-6.

(4) Romano, G.; DiBono, G. A Fatality Involving Clothiapine and Clomipramine. *J. Forensic Sci.* **1994,** *39*, 77-82.

(5) Drummer, O.H.; Horomidis, S.; Kourtis, S.; Syrjanen, M.L.; Tippett, P. Capillary Gas Chromatographic Drug Screen for Use in Forensic Toxicology. *J. Anal. Toxicol.* **1994,** *18*, 134-8.

(6) de la Torre, R.; Ortuno, J.; Pascual, J.A.; Gonzalez, S.; Ballesta, J. Quantitative Determination of Tricyclic Antidepressants and their Metabolites in Plasma by Solid-Phase Extraction (Bond-Elut T A) and Separation by Capillary Gas Chromatography with Nitrogen-Phosphorous Detection. *Therapeutic Drug Monitoring* **1998,** *20,* 340-46.

(7) Baskys, A.; Rernington, G. *Brain Mechanisms and Psychotropic Drugs,* CRC Press: New York, New York, 1996.

(8) Electronic Doctor: Medicine Information. httv://www.edoc.com(accessed April 2001)

(9) Medinfo Home Page. http://www.medinfo.co.uk (accessed April 2001)

(10) Barbey, J.T.; Rose, S.P. SSRI Safety in Overdose. J. *Clin. Psychiatry* **1998,** *59,* 42-48.

(11) Smith, P.F.; Darlington, C.L.; *Clinical Psychopharmacology,* Lawrence Erlbaum Associates, Publishers: Mahwah, New Jersey, 1996.

(12) McNair, H.M.; Miller, J. *Basic Gas Chromatography;* Wiley, John and Sons Inc. New York, New York, 1997.

(13) Start GC. http://www.members.iworld.net/suesu/ (accessed December 2000)

(14) Backer, R.C.; Pisano, R.V.; Sopher, I.M. The Comparison of Alcohol Concentrations in Postmortem Fluids and Tissues. J. *Forensic Sci.* **1980,** *25,* 327-31.

(15) Moore, K.A.; Kunsman, G.W.; Levine, B.S.; Herman, M.M.; Hyde, T.M. A Comparison of Ethanol Concentrations in the Occipital Lobe and Cerebellum. *Forensic Sci. Int.* 1997, 127-34.

(16) Caccia, S. Simultaneous Determination of Buspirone, Gepirone, Ipsapirone, and Their Common Metabolite 1-(2-Pyrimidinyl) Piperqzine in Rat Plasma and Brain by HPLC. J. *Chromatogr.,* B. 1998,431,477-80.

(17) Lai, C.T.; Tanay, VAMI; Rauw, G.A.; Bateson, A.N.; Martin, T.L.; Baker, G. B. Rapid Sensitive Procedure to Determine Buspirone Levels in Rat Brains Using Gas Chromatography with Nitrogen-Phosphorous Detection. J. *Chromatogr.,* B. 1997, *704,* 175-79.

(18) Igarashi, K.; Kasuya, F.; Fukui, M.; Abe, T.; Castagnoli, N. Jr. Simultaneous Determination of Haloperidol and its Neurotoxic Metabolite in Plasma and Brain Tissue from Schizophrenic Patients Treated with Haloperidol using HPLC and Solid-phase Extraction. ***Hochudoku* 1995,** *13, 31-8*.