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BIOEQUIVALENCE EVALUATION OF INNOVATOR AND GENERIC BUPROPION XL PRODUCTS

DIVISION OF PRODUCT QUALITY RESEARCH
OFFICE OF TESTING AND RESEARCH
CENTER FOR DRUG EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

FINAL REPORT SUBMITTED TO THE OFFICE OF GENERIC DRUGS

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INTRODUCTION

Bupropion is a widely used second generation anti-depressant. Bupropion currently has three oral tablet formulations: immediate release (dosed three times a day), sustained release (dosed twice a day) and extended release (dosed one a day). The first extended release formulation, Wellbutrin XL 300, was approved by the FDA in 2003. Budeprion XL, a generic version of the extended release bupropion formulation with a strength of 300 mg, was approved by the FDA in 2006 (http://www.fda.gov/cder/drug/infopage/bupropion/TE_review.htm.).

Shortly after Budeprion's approval, the FDA received 85 post-marketing adverse events reports from patients switching from the branded product to generic product. Among these 85 cases, 78 reported loss of antidepressant effect. The patients and physicians firmly believed that the side effects (including headache, gastrointestinal disorder, fatigue, and anxiety) and lack of efficacy were caused by poor performance of Budeprion XL. Their belief was supported by improvement of depression and lessening of side effects when the patients were switched back to Wellbutrin XL. Concerns about generic bupropion have also been the subject of news media attention (ABC News, 2007; Wall Street Journal, 2008, and NY Times 2009).

In 2008, the FDA provided the following statement after reviewing a six-month bioequivalence study on the 150 mg strength of Budeprion XL compared to Wellbutrin XL: "Although there are small differences in the pharmacokinetic profiles of these two formulations, they are not outside the established boundaries for equivalence nor are they different from other bupropion products known to be effective. The recurrent nature of major depressive disorder offers a scientifically reasonable explanation for the reports of lack of efficacy following a switch to a generic product" (FDAWebview 10/3/2008). It should be noted that the FDA did not require the sponsor (Impax) of Budeprion XL to perform a bioequivalence study on their 300-mg tablet, the most prescribed strength and the one associated with adverse events, due to potential risk of seizures at higher doses.

In light of the continuing concerns about the bioequivalence of Budeprion XL 300 mg, FDA undertook a randomized, two-treatment, crossover bioequivalence study to compare Budeprion XL 300 mg and Wellbutrin XL 300 mg tablets in healthy subjects under fasting conditions.

STUDY DESIGN AND CONDUCT

Study Materials:

The innovator and generic drug products (Table 1) of 300-mg extended-release bupropion hydrochloride tablets were purchased from commercial sources (Bradley Drugs-Washington Wholesale Drug Exchange, Bethesda, MD).

Table 1: Bupropion Drug Products

Drug Name	Wellbutrin XL	Budeprion XL
Manufacturer	Biovail Corp.	Impax Lab., Inc.
Distributor	GlaxoSmithKline	Teva Pharma.
Clinical Lot#	10C084P	9051021
Expiry Date	06/2011	05/2011
Potency (mg)	306,0	299.8

Study Dose:

Study dose is 300 mg. Bupropion is a commonly used antidepressant. Its only major complication is dose-related seizures, which usually occur only at higher doses, such as 450 mg, and then only at a rate of 1 in 1000 patients. 300 mg is considered a safe dose for the study.

Study Design:

A randomized, two-treatment, crossover bioequivalence study was used. Subjects receive a single dose of test and reference products on separate occasions with random assignment to the two possible sequences of product administration. Treatments are separated by a washout period of 15 days.

Study Subject:

Twenty four healthy subjects were used for the study. Subject demographic information is shown below.

Table 2. Subject demographic information

Age		Body Mass Index		Age Groups		Gender		Race	
				Range	%	Sex	%	Category	%
				<18	0			Caucasian	75.0
Mean	34.9	Mean	25.4	18-40	63.9	Male	61.1	African American	19.4
SD	11.3	SD	3.0	41-64	36.1	Female	38.9	Hispanic	2.8
Range	18-58	Range	19.3-31.4	65-75	0			Asian	0
				>75	0			Others	2.8

Study Conduct:

Consistent with the FDA RIHSC and Texas Tech University Health Science Center's (TTUHSC) IRB protocol, the clinical part of the study was carried out at TTUHSC and the bioanalytical assay of the bupropion plasma samples was done at the DPQR laboratories.

1. During the screening visit, eligibility requirements were confirmed and the informed consent was reviewed with the subject by a member of the study team. If the subject was eligible and agreed to participate, the subject was asked to sign and date an informed consent document prior to any study procedures. The study staff then scheduled a time for the subject's return visit to Northwest Texas Healthcare Systems (NWTHS) where the first phase of the study was being conducted.
2. For first 8 hours preceding each study visit (until the final blood draw), the subject were instructed to avoid consuming the following:
    - Alcohol
    - Grapefruit juice or whole grapefruits
    - Caffeine
    - Foods/fluids containing xanthine
3. Subjects were asked to fast for at least 10 hours prior to each of the two visits in which the study drug was administered. Subjects were allowed to drink water up until one hour before the drug administration. Female subjects were asked to use a form of birth control other than oral contraception, contraception implant, and Depo Provera shots throughout the course of the study.
4. At the return visit (to TTUHSC), a full history and physical were completed by the attending physician.
5. A urine pregnancy test was performed for each female subject prior to each study drug administration (and also at the end of the study). IF the result was positive for pregnancy, the study team would encourage the subject to seek follow up care with the appropriate health care provider.
6. NWTHS administration has approved the use of an unoccupied room at NWTHS on the 4th floor, Oncology Department. In case of varying room; CRU staff will contact NWTHS personnel for the room number in preparation for each subject's first visit. The subject remained in the NWTHS room with the TTUHSC study nurse for the first 24 hours of the study.
7. The CRU nurse or a part-time RN did the following:
    - Took vital signs- (height, weight, temperature, pulse, respirations and blood pressure
    - Placed an IV line with saline drip
    - Drew an initial sample of blood (6 mL), at pre-dose Hour 0
8. The TTUHSC nurse then administered a single Wellbutrin extended release tablet and stay with the subject in the NWTHS room for the first 24 hours. If emergency care was necessary, one of the co-investigators (TTUHSC physicians) would be consulted. All co-investigators on this study had privileges at NWTHS. If the co-investigator determines emergency care is required, the subject will be referred/admitted to NWTHS.

9. The subject consumed 240 mL (8 fluid ounces) of water with the study drug.
10. No food was allowed for at least 4 hours post-dose.
11. Water was allowed as desired, except for one hour after drug administration.
12. After the drug administration, the study nurse will collect blood (6 mL for each draw) at hours 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, and 72 hours. With the first blood draw, an IV line was placed to keep the vein open (KVO), avoiding numerous venipunctures. The study nurse will maintain this IV line with a saline drip throughout the 24-hour stay following the study drug administration; the IV rate will be KVO. The IV line was removed by the nurse when the 24 hour sample wass drawn. Subsequent blood samples on return study visits 36, 48, 60, and 72 hours (post pill), were taken by a phlebotomist or nurse at TTHUSC .
13. The samples were collected using Vacutainers® containing potassium-EDTA as the anticoagulant. Samples were centrifuged at 3000 rpm for > 10 minutes at 4 degrees Celsius. The collected plasma from each tube was placed into polypropylene cyrotubes. The samples were stored on dry ice within 2 hours of collection. At the earliest opportunity, the study nurse transported the samples from the NWTHS room to the CRU's -80 freezer. Periodically, when enough samples have been collected for a shipment, they were sent (the CRU Nurse and Pediatric Research Assistant have both completed IATA training) frozen (on dry ice) to the laboratory coordinator at the FDA. The shipment was sent overnight by FedEx. All samples were de-identified prior to being sent to the FDA.
14. Subjects received standardized meals at post-dose hours 4.5 and 9.5. NWTHS cafeteria provided the meal from their in-patient menu. An example meal would be one fried egg, one slice of Canadian bacon, one buttered English muffin, one serving of hash brown potatoes, one slice of American cheese, eight ounces of whole milk, six ounces of orange juice = 790 calories, with 17% of the calories from protein, 34% from carbohydrate, and 49% from fat. Meals/beverages will be free of grapefruit products, xanthine, and caffeine.
15. A washout period of 15 days was required between treatments, after which, this same process was followed for the generic bupropion tablets in the same group of health subjects.
16. Upon conclusion of the bupropion cycle at the end of the full study, the study nurse obtained a final urine sample from females for the pregnancy test.

Safety Monitoring

Twenty-four hour medical supervision was provided by personnel qualified to provide emergency medical care during confinement. Use of prescription medication, oral contraception, or contraception implants was not allowed within 30 days of drug administration, and depot injection of a progestogen drug was not allowed within 1 year of drug administration. A urine pregnancy test was performed for each female subject prior to drug administration in each period and at the conclusion of the study. Vital signs (blood pressure and pulse) were monitored at pre-dose Hour 0 and post-dose Hours 3, 5, 8, and 24, and at the discretion of the research staff. If emergency care was necessary, one of the co-investigators (TTUHSC physicians) was consulted.

All co-investigators on this study had privileges at NWTHS. If the co-investigator determined emergency care is required, the subjects were referred/admitted to NWTHS. If a subject experienced a significant side effect due to the drug, the subject was required to remain in the hospital for a period of up to 3 days or until the symptoms cleared.

Reporting

The following adverse events were monitored.

:

- Headache (including migraine)
- Infection
- Abdominal pain
- Asthenia
- Chest pain
- Pain
- Fever
- Hot flashes
- Dry mouth
- Palpitation
- Flushing
- Hypertension
- Hypotension
- Nausea
- Constipation
- Diarrhea
- Anorexia
- Vomiting
- Dysphagia
- Appetite increase
- Dyspepsia

STUDY SUMMARY

Table 3: Fasting Bioequivalence Study Design Summary

Study Summary, Fasting Bioequivalence Study	
Study No.	A10-3574
Study Design	Open label, randomized, single-dose, two-treatment, two-period, two-sequence crossover study under fasting conditions.
No. of subjects enrolled	27
No. of subjects completed	24
No. of subjects analyzed	24
Subjects (Healthy or Patients	Healthy
Sex(es) included	Male: 9 Female: 15
Test product	BUDEPRION XL (Bupropion Hydrochloride) Extended Release Tablets
Reference product	WELLBUTRIN XL® (Bupropion Hydrochloride) Tablets
Strength tested	300 mg tablet
Dose	1 x 300 tablet with approximately 240 mL of water under fasting condition

BIOANALYTICAL METHOD DEVELOPMENT

The purpose of this study was to develop and validate a bioanalytical method to determine the plasma drug concentration of bupropion following oral administration of the innovator and generic extended release drug products. Bupropion is the water soluble hydrochloride salt of a monocyclic aminoketone used clinically to treat depression (Schroeder, 1983). Bupropion has additional clinical indications including smoking cessation, attention deficit hyperactivity disorder, obesity, and seasonal affective disorder (Wilens, 2001). O'Byrne (2010) notes that in 2007 bupropion, a second-generation anti-depressant was the fourth most prescribed anti-depressant in the US.

PROJECT OBJECTIVE

To develop and implement a Ultra-Performance Liquid Chromatograph-Mass Spectrometry (UPLC-MS) method for the evaluation of bupropion and its metabolites in human plasma to evaluate the bioequivalence of innovator and generic XL drug products.

OVERALL OBJECTIVES OF THE STUDY

An ultra-performance liquid chromatography (UPLC) mass spectrometry (MS) method was developed and validated to assay bupropion and its major metabolite hydroxyl bupropion and its two minor metabolites erythro- and threo- bupropion. The results of this validated UPLC-MS method will be used:

(i) to evaluate the bioequivalence (BE) of commercially available innovator and generic extended release products of bupropion.
(ii) to study the metabolism of hydroxy bupropion from innovator and generic extended release products of bupropion.
(iii) to study the metabolism of erythro- and threo- bupropion from innovator and generic extended release products of bupropion

BACKGROUND

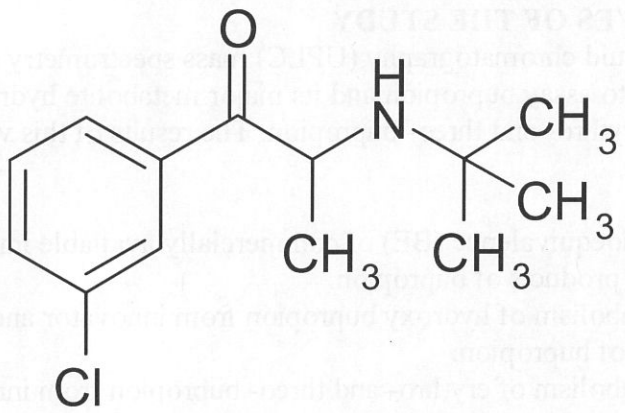
Bupropion free base

Bupropion free base (Figure 1) has a molecular formula of $C_{13}H_{18}CINO$. The molecular weight of bupropion is 239.74, and its CAS Number is 34911-55-2. The percent elemental composition of bupropion free base is: Carbon 65.13%, Hydrogen 7.57%, Nitrogen 5.84%, Oxygen 6.67%. Bupropion's CAS name is 1-(3-Chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone. [Merck Index]

Log P and pKa

The distribution coefficient logP at 37°C in n-octanol water solution pH 7 is 3.4. The pKa is 7.9. The chemical structure of bupropion free base is shown in Figure 1.

Figure 1: Chemical structure of bupropion free base



Bupropion hydrochloride

Bupropion hydrochloride (Figure 2) has a molecular formula of $C_{13}H_{18}CINO.HCl$. The molecular weight of bupropion hydrochloride is 276.20, and its CAS Number is 31677-93-7.

Bupropion hydrochloride is a white powder that is soluble in dilute 0.01 M hydrochloric acid and very soluble in ethanol, acetonitrile, n-octanol and ethyl acetate. Bupropion hydrochloride was found to be stable in 0.01 M hydrochloric acid.

Bupropion (Mehta, 1983) also known as amfebutamone has been identified as a dopamine and norepinephrine reuptake inhibitor (Stahl, 2004). Bupropion is metabolized to three active metabolites: hydroxy bupropion (the major metabolite) and erythro- and threo- bupropion, in humans. Bromo bupropion, a structural analog, was selected as the internal standard for this bioanalytical study.

The chemical structures of bupropion hydrochloride, erythro bupropion, threo bupropion, and bromo bupropion are shown below in Figure 2-6.

Figure 2: Chemical structure of bupropion hydrochloride

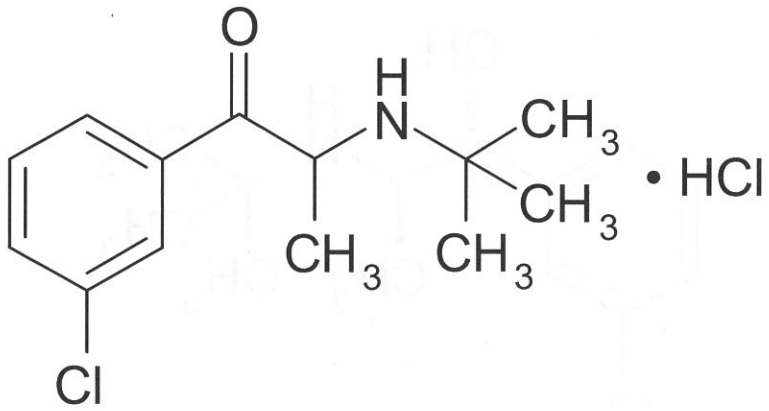


Figure 3: Chemical structure of hydroxy bupropion

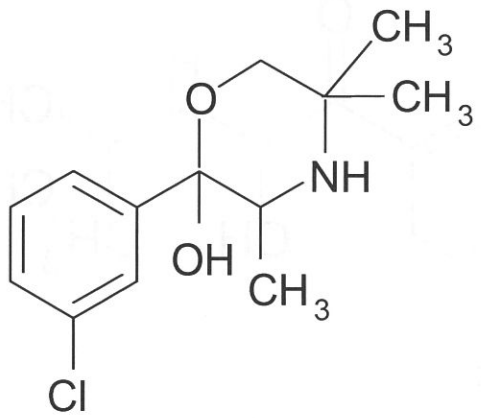


Figure 4: Chemical structure of erythro bupropion

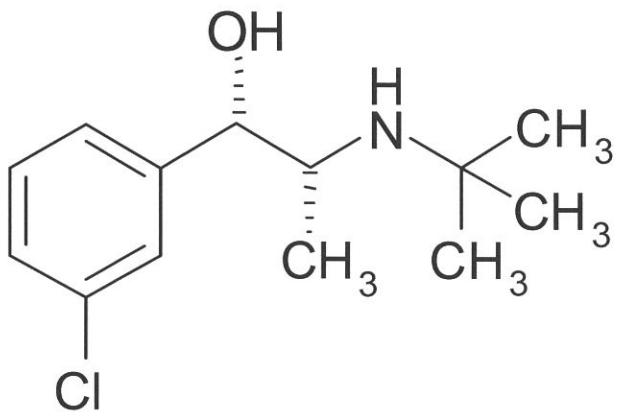


Figure 5: Chemical structure of threo bupropion

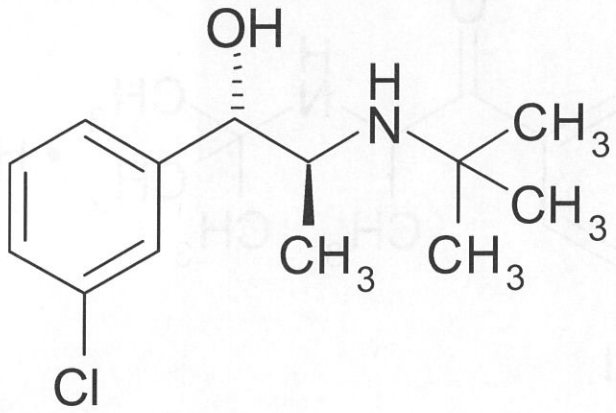
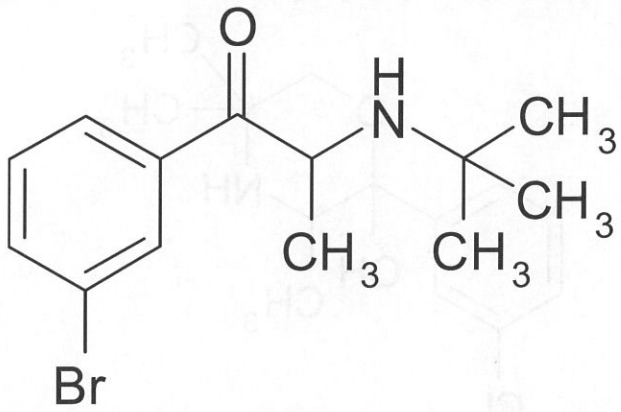


Figure 6: Chemical structure of bromo bupropion (internal standard)



MATERIALS AND METHODS

Drug Substance

Bupropion hydrochloride (product number 1078733, lot H0G179) and bromo bupropion (product number 1078755, lot F0E011) hydrochloride reference standards were purchased from The United States Pharmacopoeia Convention, Inc., (Rockville, MD). Erythro bupropion (product number D448650, lot 1-SHG-40-1) and threo bupropion (product number D448675, lot 1-SGH-39-2) reference standards were purchased from Toronto Research Chemicals (TRC). All standards purchased were supplied with certificates of analysis. Hydroxy bupropion (lot Rs-8-1) was synthesized in house at DPQR. Hydroxy bupropion was fully characterized by proton and carbon nmr spectroscopy, mass spectrometry, infra-red spectroscopy and UPLC. The purity of all standards was verified by UPLC-UV.

Chemicals and Reagents

HPLC grade acetonitrile, tetrahydrofuran (THF), hydrochloric and formic acid and potassium hydroxide were purchased from Fischer Scientific (Pittsburgh, PA). Ammonium formate USP grade (JT Baker, Phillipsburg, NJ), phosphoric acid (85%) HPLC grade (EMD Chemicals Inc., Gibbstown, NJ), sodium chloride (Fisher Chemicals, Certified for biological work) and certified pH buffers (4, 5 and 7) were purchased from Fisher Scientific (Pittsburgh, PA). Solvent nylon filters (0.45 microns) were purchased from Millipore Corporation (Bedford, MA). Filtered 18 MOhm water was supplied in house by a Millipore Milli-Q System (Bedford, MA).

Preparation of mobile phase

The mobile phase composition (7% ACN and 3.1% THF/4mM ammonium formate buffer (pH = 4.02)) was optimized after several experimental UPLC analysis with bupropion and each metabolite over their respective analytical ranges.

Ammonium formate was weighed (504.4 grams) and transferred to a 2000 mL beaker, dissolving in 2000 mL deionized water-stock solution I. A stirring bar was placed in the beaker and stirred using electric stirrer for 5 minutes. Slowly add 6 drops of 88% formic acid and checked pH with a calibrated pH meter to achieve a final pH of 4.02. Allow 15 minutes of slow stirring to verify equilibrium of pH. The pH was adjusted with dilute formic acid or potassium hydroxide as required. The buffer solution was filtered through a 0.45 micron nylon membrane filter under vacuum. The buffer was prepared daily or at time of use. 1000 mL of ammonium formate buffer was transferred to a 2000 mL volumetric and added 108.8 grams (approx. 140 mL) of acetonitrile to the 2000 mL volumetric Add 55.1 grams (approx. 62 mL) of tetrahydrofuran to the 2000 mL volumetric. Then add additional ammonium formate buffer to bring the total volume to 2000 mL to yield a mobile phase of 7% ACN and 3.1% THF/4mM ammonium formate buffer (pH = 4.02).

Sample and Standard Preparation

Bupropion hydrochloride, metabolites, the internal standard bromo bupropion, samples and standards were weighed out in diffused light and were placed in glass volumetric flasks. Sample and standard vials were wrapped in aluminum foil and handled in reduced lighting to protect from light during analytical preparation before being placed in the UPLC auto sampler.

Preparation of Stock Standard Solutions

Bupropion stock standard solutions-(1 mg/mL) and metabolites and bromobupropion the internal standard were prepared from the reference standard material in 0.01M hydrochloride acid. The free base weight of bupropion hydrochloride USP reference standard was weighed and transferred to a 10-ml low volumetric flask containing 10 ml of 0.01M hydrochloride acid. The standard stock solutions-I and II for each analyte were stored in the refrigerator at 4° C.

Preparation of working standard Solutions (System suitability, calibration and QC)

Bupropion working standard solutions-I and II were prepared from Standard stock solution-I and II respectively by diluting with mobile phase to produce a final concentration of 10 µg/ml. These working standard solutions were stored at 4°C.

Preparation of Standard solution for System Suitability

A standard solution bupropion and bromo bupropion was prepared daily by serial dilution in mobile phase from the working standard solution-I to produce a nominal concentration 25 ng/mL of bupropion and 25 ng/mL of bromo bupropion that was analyzed by UV and MS detection.

Preparation of Calibration Standards from working standard-I

Eight calibration standard solutions were prepared daily in human plasma from the bupropion working standard solution-I to produce nominal concentrations of 2.5, 5.0, 10, 25, 50, 100 and 200, and 250 ng/mL of bupropion.

Six calibration standard solutions were prepared daily in human plasma from the erythro bupropion working standard solution-I to produce nominal concentrations of 2.5, 5.0, 10, 25, 50, 100 ng/mL of erythro bupropion.

Seven calibration standard solutions were prepared daily in human plasma from the threo bupropion working standard solution-I to produce nominal concentrations of 2.5, 5.0, 10, 25, 50, 100 and 200 ng/mL of threo bupropion.

Six calibration standard solutions were prepared daily in human plasma from the hydroxy bupropion working standard solution-I to produce nominal concentrations of 10, 20, 40, 100 200 and 250 ng/mL of hydroxy bupropion for the low analytical range calibration curve (10-250 ng/mL).

Six calibration standard solutions were prepared daily in human plasma from the hydroxy bupropion working standard solution-I to produce nominal concentrations of 200, 250, 400, 500 600 and 750 ng/mL of hydroxy bupropion for the high analytical range calibration curve (200-750 ng/mL).

Preparation of Quality Control Standards

Five quality control standard solutions were prepared at each QC level in advance in human plasma from the bupropion working standard solution-II to produce nominal concentrations of 2.5, 5, 100 and 250 ng/mL of bupropion at the QC low standard (2.5 µg/mL), ≤3X QC standard (5 µg/mL), QC intermediate standard (100 µg/mL) and QC high standard (250 µg/mL).

Five quality control standard solutions were prepared at each QC level in human plasma from the erythro bupropion working standard solution-II to produce nominal concentrations of 2.5, 5, 100 and 250 ng/mL of erythro bupropion at the QC low standard (2.5 µg/mL), ≤3X QC standard (5 µg/mL), QC intermediate standard (50 µg/mL) and QC high standard (100 µg/mL).

Five quality control standard solutions were prepared at each QC level in human plasma from the threo bupropion working standard solution-II to produce nominal concentrations of 2.5, 5, 100 and 250 ng/mL of threo bupropion at the QC low standard (2.5 µg/mL), ≤3X QC standard (5 µg/mL), QC intermediate standard (100 µg/mL) and QC high standard (200 µg/mL).

Five quality control standard solutions were prepared at each QC level in human plasma from the hydroxy bupropion working standard solution-II to produce nominal concentrations of 10, 20, 100 and 250 ng/mL of hydroxy bupropion at the QC low standard (10 µg/mL), ≤3X QC standard (20 µg/mL), QC intermediate standard (100 µg/mL) and QC high standard (250 µg/mL) low analytical range calibration curve.

Five quality control standard solutions were prepared at each QC level in human plasma from the hydroxy bupropion working standard solution-II to produce nominal concentrations of 200, 250, 400 and 750 ng/mL of hydroxy bupropion at the QC low standard (200 µg/mL), ≤3X QC standard (250 µg/mL), QC intermediate standard (400 µg/mL) and QC high standard (750 µg/mL) high analytical range calibration curve.

Solid Phase Extraction (SPE) Procedure

Bupropion plasma samples were extracted from human plasma by SPE

1. Pipet 25µL of a 2µg/mL solution of bromo bupropion in 0.01N HCl into a 15 mL polypropylene conical tube.
2. Add 1mL of plasma sample, vortex for 10s, and allow to sit for 10minutes.
3. Add 1.0mL of acetonitrile to precipitate proteins. Vortex for 10s. Allow samples to sit for 5minutes and vortex again for 10s. 10minutes after adding acetonitrile, vortex a third time for 10s.
4. Centrifuge samples at 2100 x G for 10minutes.
5. Condition 500mg Agilent Bond Elut C18 columns by filling reservoir with acetonitrile followed by filling reservoir with deionized water (diH_2O). Allow liquid to flow through tube by gravity.
6. Remove 1.7mL sample supernatant and apply to SPE column. Allow liquid to flow through tube by gravity.
7. Wash SPE columns with 3mL of 25:75 acetonitrile:diH_2O. A small amount of vacuum may be applied. Flow should be ≤2mL/min.
8. Elute SPE columns with 3mL of 50:50 acetonitrile:0.01N HCl. Collect eluent in a 12 x 75mm glass tube. A small amount of vacuum may be applied. Flow should be ≤ 2mL/min.
9. Dry eluent in the Speed vacuum concentrator without heat.

10. Reconstitute samples in 375µL UPLC mobile phase (89.9:7:3.1 4mM Ammonium Formate (pH=4.0):Acetonitrile:tetrahydrofuran) by vortexing for 15seconds.
11. Transfer reconstituted liquid to a microcentrifuge tube and centrifuge in the eppendorf centrifuge at 14000RPM for 10 minutes.
12. Transfer 250µL supernatant to a 300µL polypropylene UPLC mass spectroscopy vial for UPLC injection.

High-Performance Liquid Chromatography System

A Waters Acquity Series Ultra-Performance Liquid Chromatography (HPLC) system equipped with binary solvent pump, in-line degassing, autosampler, photodiode array detector, thermostated column compartment and Empower 2 chromatographic software was used for bupropion analysis.

Separation was achieved on an Acquity BEH reverse-phase C18, 100 x 2.1 mm column with 1.7 mm particle size placed in series with an Acquity BEH reverse-phase C18, guard column (20 X 2.1 mm) with 1.7 mm particle size. All experiments were carried out at 35°C and at a flow rate of 0.5 mL/min mobile phase with an injection volume of 10 mL under full loop conditions. Mobile phase was degassed with on-line degasser and delivered isocratically containing 4.0 mM Ammonium Formate buffer at 7% ACN and 3.1% THF (pH = 4.02).

Mass Spectrometer System

A Waters corporation SQD 3100 mass spectrometer interfaced with the UPLC was operated in positive ion mode with Electro Spray Ionization source temperature of 150°C, Desolvation temperature 400 °C, Desolvation gas flow 700-800 L/hr, and Cone Voltage of 22.3 (analyte-specific parameter). Control and data acquisition was accomplished with Waters Empower™ chromatographic instrument was recorded with the computer control software

The UPLC-Electro Spray ionization mass spectrometry method was conducted in SIR (Single Ion Recording) mode to record Bupropion response at an atomic mass of 240.2 amu, Hydroxy Bupropion-256.1 amu, Erthro Bupropion and Threo Bupropion at 242.09 amu and the internal standard Bromo Bupropion at 284.5 amu.

UPLC Analysis

Standards and samples (1 mL) were transferred to UPLC vials, capped and placed in an automated HPLC injector module. Samples were injected in an automated fashion onto the UPLC and chromatographically analyzed using Waters Corporation Acquity C-18 BEH UPLC column technology. Chromatographic and mass spectrometry data for the bupropion dissolution samples was collected by the UPLC computer using Empower 2 chromatographic software.

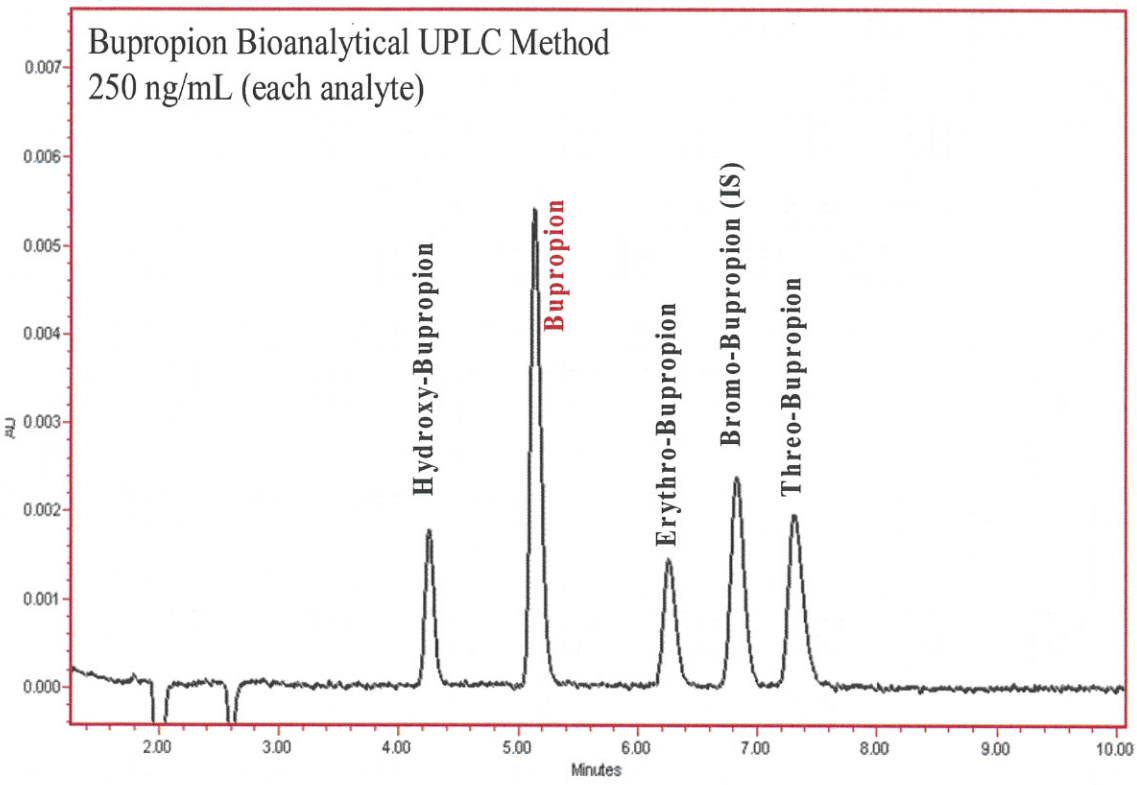
UPLC Method

The analytical method for Bupropion and metabolites was developed in house by DPQR.

Table 4: General operating parameters and description of the UPLC method

UPLC system	Water Corporation Acquity
Chromatographic Software	Water Corporation Empower 2
Detection	UV @ 212 nm
Column	Water Corporation Acquity BEH C-18 100 × 2.1 mm, 1.7μ
Guard Column	Water Corporation Acquity BEH C-18 20 × 2.1 mm, 1.7μ
Mobile phase	2 mM Ammonium formate (7% ACN/3.1% THF)
Elution	Isocratic
pH	4.02
Flow rate	0.5 mL/min
Column temperature	35 °C
Injection volume	10 μL

Figure 7: Representative Chromatogram

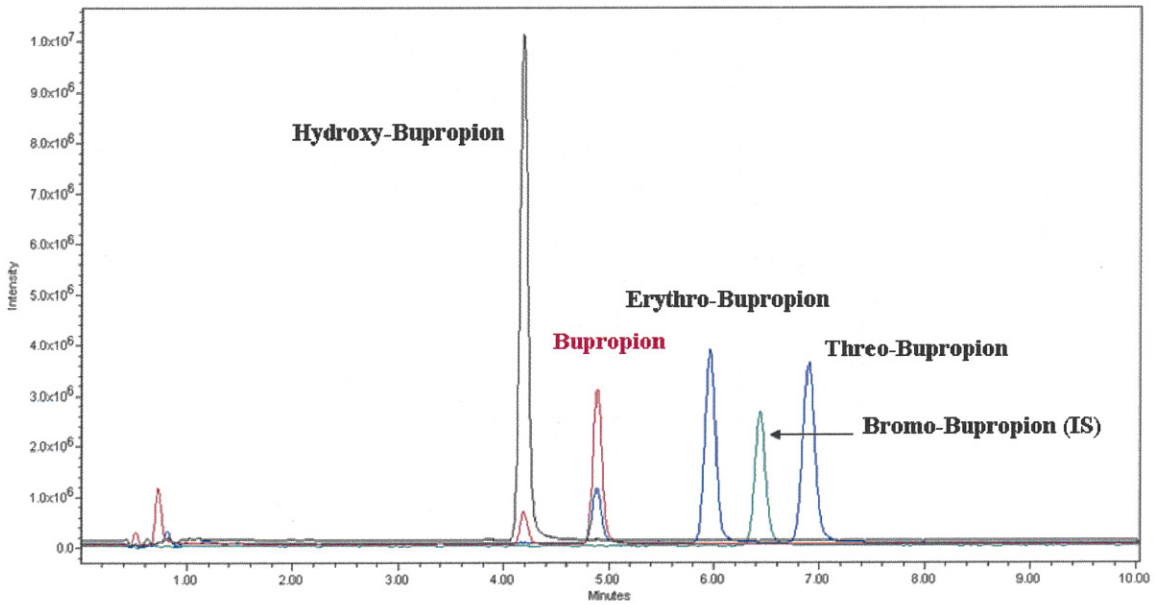


UPLC-MS Method

Table 5: General operating parameters and description of the UPLC-MS method

UPLC system	Water Corporation Acquity
Chromatographic Software	Water Corporation Empower 2
Mass Spectrometer	Water Corporation SQD
Mode	Positive ion
Ionization	Electrospray
Desolvation temperature	400 °C
Desolvation gas flow	800 L/hr
Cone Voltage	22-30 V
Detection mode	SIR (Single Ion Recording) mode
Bupropion response	240.20 atomic mass units (amu),
Erthro Bupropion response	242.09 amu
Threo Bupropion response	242.09 amu
Hydroxy Bupropion response	256.10 amu
Bromo Bupropion response	284.50 amu

Figure 8: Mass chromatogram of bupropion and metabolites



BIOANALYTICAL METHOD VALIDATION

The analytical method was validated according to FDA Bioanalytical Method Validation accuracy, precision, linearity, selectivity, analytical range and stability the USP <621> for Chromatography.

Table 6: Bioanalytical Method Validation Parameters

Validation Parameters	Acceptance Criteria
System Suitability (n = 6)	
RSD (%) of retention time	<2%
RSD (%) of Peak area	<2%
Average of k`	>4.0
USP Tailing factor	<2.0
Average of column plates	>8000
Averae of peak symmetry	>0.5
***Linearity of Calibration Curves (n=2)**	> 0.99
Accuracy	
Low QC (n = 5)	80-120%
3X Low QC (n =5)	95-115%
Intermediate QC (n = 5)	95-115%
High QC (n = 5)	95-115%
Precision	
Low QC (n = 5)	<15.0%
3X Low QC (n =5)	<15.0%
Intermediate QC (n = 5)	<15.0%
High QC (n = 5)	<15.0%
Analytical Range (bupropion)	2.5-250 ng/mL
Analytical Range (H-bupropion-low)	10-250 ng/mL
Analytical Range (H-bupropion-high)	200-750 ng/mL
Analytical Range (E-bupropion)	2.5-100 ng/mL
Analytical Range (T-bupropion)	2.5-200 ng/mL

*H-bupropion high level specification was set to >0.95.

Sample Calculation

A linear calibration model was generated as a weighted (1/Y) least squares fit of measured peak areas (Y) to known calibration sample concentrations (X). The resulting weighted linear function was used to calculate the concentration of Bupropion for the study sample or quality control standards from assayed peak areas.

Accuracy and precision are calculated from the concentration data and the peak response of the quality control standards using the weighted linear function.

Analytical range is established by determining that the accuracy, precision and linearity are acceptable over the analytical range according to the ICH Q2B.

Specificity is determined by the observation of no endogenous peaks in the sample blanks or no co-eluting peaks in the sample or calibration standards and comparison of the sample to the known reference standard.

RESULTS

UPLC Assay Method

The analytical method for bupropion was validated according to the requirements of the FDA Bioanalytical Method Validation Guidance for Industry and was determined to be accurate, precise, specific and linear over the established analytical range for bupropion, hydroxy bupropion, erythro bupropion and threo bupropion.

Table 7: System suitability results

Acceptance Criteria	**Specifications**	**Day 1**	**Day 2**	**Day 3**	**Passed/ failed**
RSD (%) of retention time	**<5%**	0.024	0.025	0.023	Passed
RSD (%) of Peak area	**<2%**	1.90	1.33	0.99	Passed
Average of k'	**>4.0**	9.47	9.28	9.36	Passed
Average of USP Tailing factor	**<2.0**	1.19	1.18	1.13	Passed
Average of column plates	**>8000**	12520	12243	13,165	Passed

Table 8: Linearity of calibration curves

Validation Summary -- Linearity -- Days 1 to 3					
	Analytic Range	Levels	Slope	Intercept	R^2
Bupropion	2.5 to 250 ng/mL	8	0.1029	0.0407	0.9988
			0.1029	0.0393	0.9996
			0.1041	0.0534	0.9992
Erythro Bupropion	2.5 to 100 ng/mL	6	0.1528	0.0167	0.9996
			0.1469	0.0216	0.9977
			0.1627	0.0713	0.9919
Threo Bupropion	2.5 to 200 ng/mL	7	0.1546	0.0273	0.9988
			0.1483	0.0662	0.9990
			0.1668	0.1088	0.9986
Hydroxy Bupropion	10 to 250 ng/mL	6	0.0721	0.1583	0.9988
			0.0714	0.1562	0.9983
			0.0765	0.2343	0.9956
Hydroxy Bupropion	200 to 750 ng/mL	6	0.0492	5.1197	0.9966
			0.0592	2.5550	0.9950
			0.0541	5.3138	0.9652

Table 9: Accuracy results-method validation

ACCURACY	QC LEVEL	SPECIFICATIONS	DAY-1	DAY-2	DAY-3	PASSED/ FAILED
Accuracy- Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**80-120%**	87.3	98.0	87.1	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**85-115%**	90.9	98.0	96.4	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**85-115%**	103.5	103.7	102.3	***Passed***
High QC (n = 5)	**250 ng/mL**	**85-115%**	95.9	102.8	95.6	***Passed***
Accuracy-Hydroxy Bupropion (low range)						
Low LOQ QC (n = 5)	**10 ng/mL**	**80-120%**	93.3	93.0	83.4	***Passed***
<3 X LLOQ QC (n = 5)	**20 ng/mL**	**85-115%**	100.1	102.5	98.7	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**85-115%**	105.1	104.3	103.6	***Passed***
High QC (n = 5)	**250 ng/mL**	**85-115%**	94.8	102.3	94.3	***Passed***
Accuracy-Hydroxy Bupropion (high range)						
Low LOQ QC (n = 5)	**200 ng/mL**	**80-120%**	94.9	104.2	100.3	***Passed***
<3 X LLOQ QC (n = 5)	**250 ng/mL**	**85-115%**	98.2	106.8	97.5	***Passed***
Intermediate QC (n = 5)	**400 ng/mL**	**85-115%**	98.8	102.8	98.4	***Passed***
High QC (n = 5)	**750 ng/mL**	**85-115%**	93.2	99.1	93.7	***Passed***
Accuracy-Erythro Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**80-120%**	91.5	100.4	90.5	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**85-115%**	92.2	101.6	98.9	***Passed***
Intermediate QC (n = 5)	**50 ng/mL**	**85-115%**	100.4	101.6	104.8	***Passed***
High QC (n = 5)	**100 ng/mL**	**85-115%**	98.9	101.5	98.0	***Passed***
Accuracy-Threo Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**80-120%**	91.6	90.5	86.1	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**85-115%**	92.5	98.5	95.8	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**85-115%**	103.2	104.6	101.6	***Passed***
High QC (n = 5)	**200 ng/mL**	**85-115%**	96.5	100.4	96.5	***Passed***

The analytical method was determined to be accurate at each quality control level and met the acceptance criteria.

Table 10: Precision results-method validation

PRECISION	QC LEVEL	SPECIFICATIONS	DAY-1	DAY-2	DAY-3	PASSED/ FAILED
Precision- Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**< 20%**	**1.61**	**9.38**	**3.53**	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**<15%**	**1.86**	**0.49**	**2.04**	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**<15%**	**1.27**	**1.42**	**0.86**	***Passed***
High QC (n = 5)	**250 ng/mL**	**<15%**	**0.45**	**0.55**	**1.28**	***Passed***
Precision-Hydroxy Bupropion (low range)						
Low LOQ QC (n = 5)	**10 ng/mL**	**< 20%**	**1.87**	**3.48**	**2.13**	***Passed***
<3 X LLOQ QC (n = 5)	**20 ng/mL**	**<15%**	**1.95**	**1.70**	**1.90**	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**<15%**	**1.03**	**2.04**	**10.50**	***Passed***
High QC (n = 5)	**250 ng/mL**	**<15%**	**1.41**	**1.60**	**2.48**	***Passed***
Precision-Hydroxy Bupropion (high range)						
Low LOQ QC (n = 5)	**200 ng/mL**	**< 20%**	**2.02**	**2.43**	**10.23**	***Passed***
<3 X LLOQ QC (n = 5)	**250 ng/mL**	**<15%**	**1.99**	**1.85**	**3.37**	***Passed***
Intermediate QC (n = 5)	**400 ng/mL**	**<15%**	**2.58**	**2.68**	**4.59**	***Passed***
High QC (n = 5)	**750 ng/mL**	**<15%**	**1.88**	**2.61**	**1.48**	***Passed***
Precision-Erythro Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**< 20%**	**1.25**	**3.15**	**2.37**	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**<15%**	**2.61**	**1.79**	**1.92**	***Passed***
Intermediate QC (n = 5)	**50 ng/mL**	**<15%**	**1.44**	**1.18**	**5.02**	***Passed***
High QC (n = 5)	**100 ng/mL**	**<15%**	**0.78**	**1.24**	**1.39**	***Passed***
Precision-Threo Bupropion						
Low LOQ QC (n = 5)	**2.5 ng/mL**	**< 20%**	**2.21**	**6.12**	**4.94**	***Passed***
<3 X LLOQ QC (n = 5)	**5.0 ng/mL**	**<15%**	**1.64**	**3.63**	**4.41**	***Passed***
Intermediate QC (n = 5)	**100 ng/mL**	**<15%**	**1.22**	**1.24**	**0.41**	***Passed***
High QC (n = 5)	**200 ng/mL**	**<15%**	**0.93**	**0.81**	**1.00**	***Passed***

The analytical method was determined to be precise at each quality control level and met the acceptance criteria.

Range

Analytical range was established by demonstrating acceptable ***accuracy, precision and linearity*** of the method over the analytical range for bupropion, hydroxy bupropion, erythro bupropion and threo bupropion.

Table 11: With-in study quality control and calibration standards-bupropion

	Bupropion							
QC Conc. (ng/mL)	5	50	100	200	250			
Inter day Precision (%CV)	3.49	2.48	1.99	2.05	3.24			
Inter day Accuracy (%)	98.10	102.62	101.47	99.53	99.54			
Cal. Standards Conc. (ng/mL)	2.5	5	10	25	50	100	200	250
Inter day Precision (%CV)	6.88	2.77	2.24	2.55	2.29	1.29	2.02	1.70
Inter day Accuracy (%)	97.30	97.63	100.94	101.93	102.49	101.26	99.96	99.02
Linearity Range (range of R^2)	0.9976 - 1.0000							

Table 12: With-in study quality control and calibration standards -erythro bupropion

	Erythro-Bupropion					
QC Conc. (ng/mL)	5	50	100			
Inter day Precision (%CV)	3.01	2.50	1.73			
Inter day Accuracy (%)	100.17	102.69	99.33			
Cal. Standards Conc. (ng/mL)	2.5	5	10	25	50	100
Inter day Precision (%CV)	2.84	2.12	1.48	2.14	1.78	0.97
Inter day Accuracy (%)	95.90	99.51	102.45	102.25	101.44	98.72
Linearity Range (range of R^2)	0.9980 - 1.0000					

Table 13. With-in study quality control and calibration standards -threo bupropion

	Threo bupropion							
QC Conc. (ng/mL)	5	50	100	200				
Inter day Precision (%CV)	2.19	2.65	2.12	2.11				
Inter day Accuracy (%)	98.96	104.49	102.02	98.13				
Cal. Standards Conc. (ng/mL)	2.5	5	10	25	50	100	200	
Inter day Precision (%CV)	5.09	2.38	1.92	2.86	2.40	1.16	1.06	
Inter day Accuracy (%)	91.59	98.78	102.59	103.94	103.84	101.47	98.01	
Linearity Range (range of R^2)	0.9980 - 0.9999							

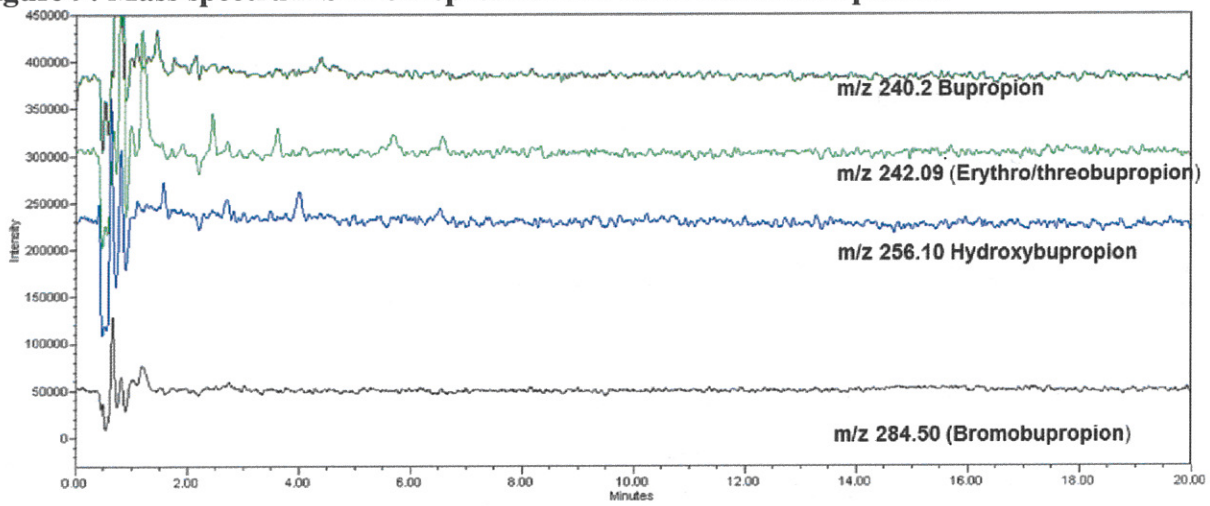
Table 14: With-in study quality control and calibration standards -hydroxy bupropion

	Hydroxy bupropion						
QC Conc. (ng/mL)	20	100	250	400	750		
Inter day Precision (%CV)	6.78	2.17	2.40	3.06	4.630379		
Inter day Accuracy (%)	101.51	105.14	99.25	103.93	97.34597		
	Lower Range						
Cal. Standards Conc. (ng/mL)	10	20	40	100	200	250	
Inter day Precision (%CV)	5.53	3.78	2.84	2.61	1.51	1.42	
Inter day Accuracy (%)	89.44	101.77	105.66	106.00	100.22	97.22	
Linearity Range (range of R^2)	0.9944 - 0.9998						
	Upper Range						
Cal. Standards Conc. (ng/mL)	200	250	400	500	600	750	
Inter day Precision (%CV)	3.08	2.04	4.77	3.02	2.28	2.53	
Inter day Accuracy (%)	95.51	99.46	104.41	103.10	101.82	96.13	
Linearity Range (range of R^2)	0.9628 - 0.9996						

Selectivity

Selectivity was verified by the observation (Figures 9) of no endogenous peaks in the mobile phase, plasma blanks or no co-eluting peaks in the sample or calibration standards were observed by UV or mass detection. Ten different lots of blank human plasma was tested and six patient plasma zero hour samples were tested at the atomic mass of each of the analyte.

Figure 9: Mass spectrum SIR of representative lot of blank NIH plasma



Selectivity was also verified by the observation that commonly used medication such as aspirin, acetaminophen, ibuprofen and caffeine and nicotine did not co-elute with the analytes of interest.

In summary, selectivity was verified by the observation of no endogenous peaks or identified bupropion, earthier threo bupropion, hydro bupropion or bromo bupropion peaks in 10 lots of blank NIH plasma samples and six patient zero hour sample at each analyte's respective amu.

Matrix Effect-Ion Suppression

Figure 10: Chromatogram of post-column infusion experiment using single ion monitoring of Bupropion (m/z = 240.20, RT 4.80 min) using positive mode electrospray at optimized conditions

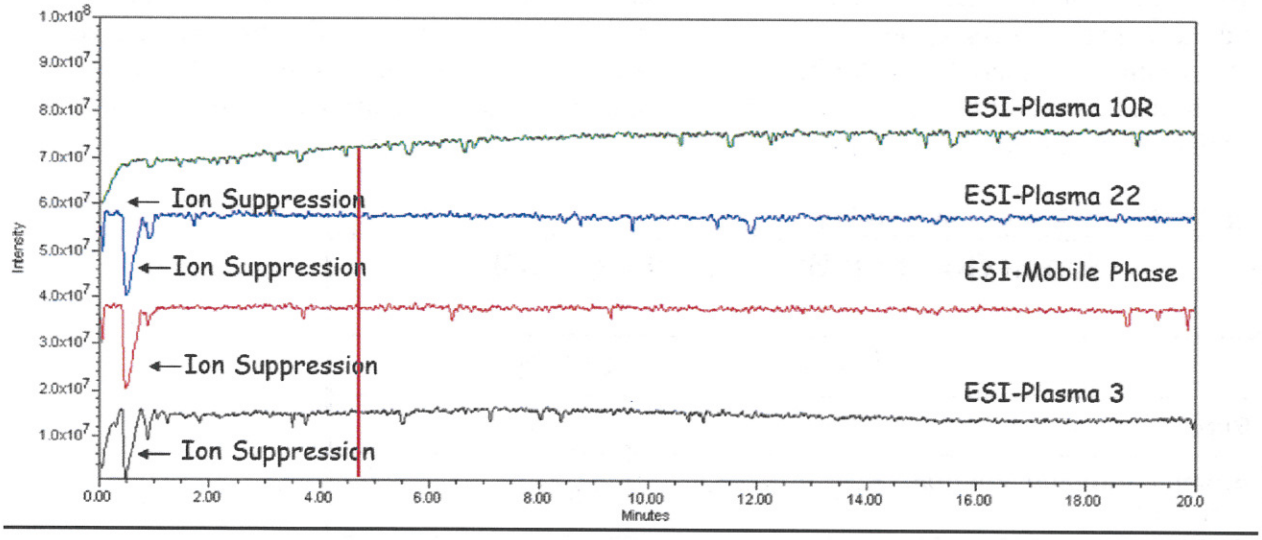
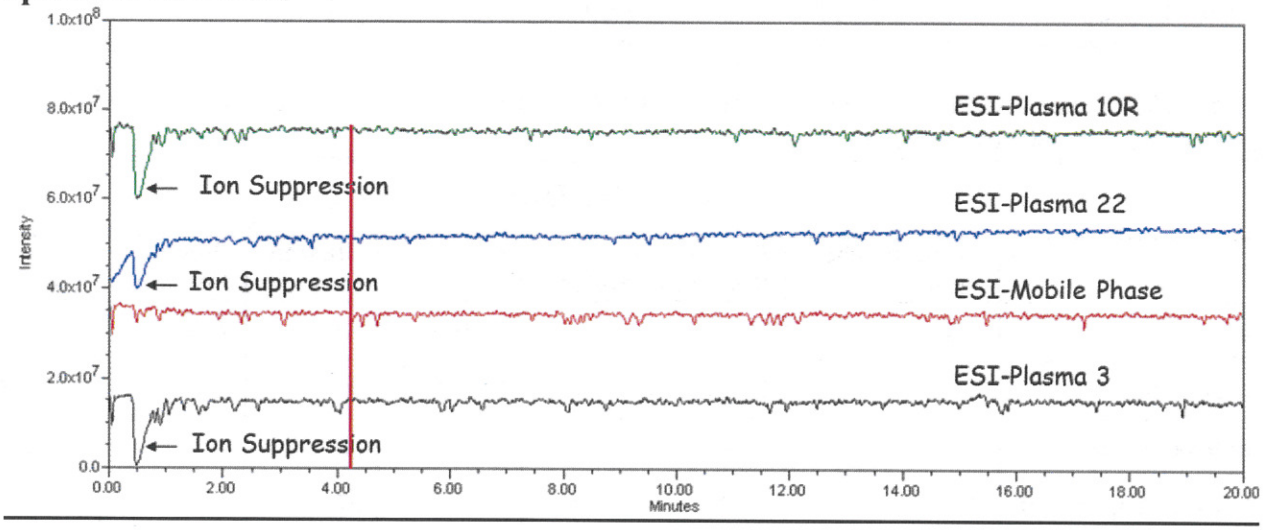


Figure 11: Chromatogram of post-column infusion experiment using single ion monitoring of Erthyro bupropion (m/z = 242.09, RT 5.70 min) using positive mode electrospray at optimized conditions



The matric effect experimental results were similar for hydro bupropion, threo bupropion and bromo bupropion at 4.3, 6.3 and 6.6 minutes respectively. In summary there was no observed matrix effect from the mobile phase or from the multiple lots of human plasma tested.

Bioanalytical Stability

Stability studies were conducted according to the FDA Bioanalytical Guidance for Industry. As defined by the guidance, drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. Stability procedures should evaluate the stability of the analytes during sample collection and handling, and the analytical parts of the procedure. All stability studies used, according to the guidance, sets of samples prepared from freshly made stock solution of the analyte/s (parent drug and metabolites) at the LLOQ and the highest calibration standard concentration. Blank plasma was obtained from the NIH blood bank.

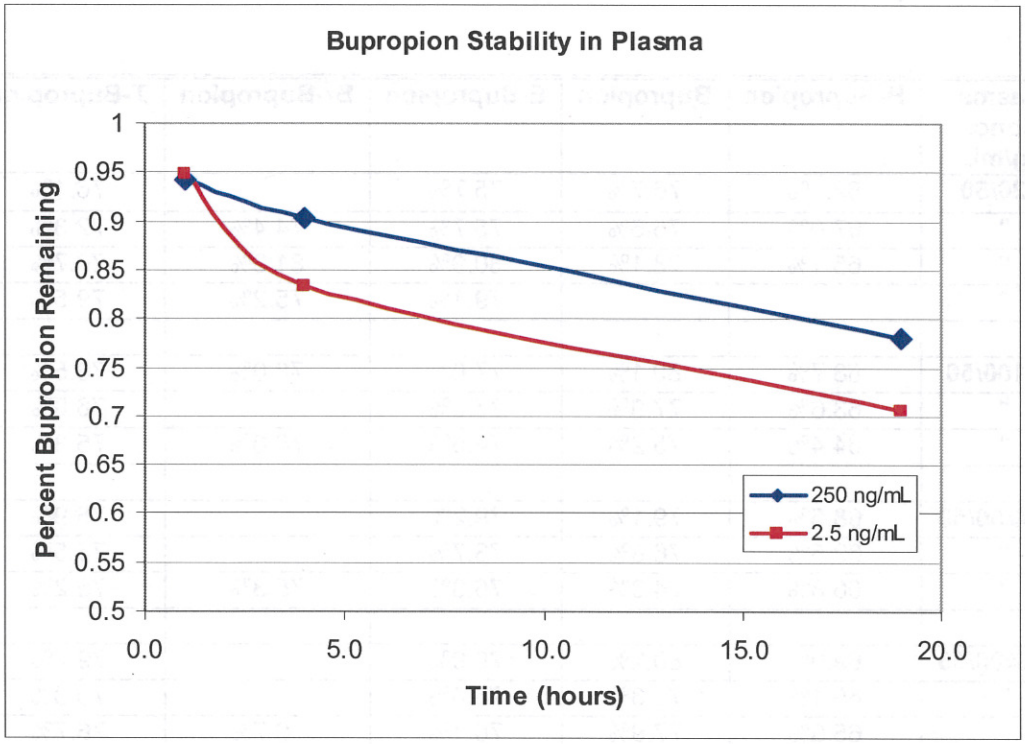
Table 15: Stock solution stability

Stock Solution Stability, 0.01N HCl		
	Time (days)	Stability
Bupropion	188	101.4%
Bromo bupropion (Internal Standard)	84	100.4%
Hydroxy bupropion	168	102.6%
Erythro bupropion	168	97.8%
Threo bupropion	168	99.1%

Table 16: Freeze and thaw plasma stability, short term room temperature plasma stability and post preparation stability

Stability Data						
	Concentration (ng/mL)	Freeze-Thaw (Plasma-3 cycles)	Plasma (1-hour)	Plasma (4-hour)	Plasma (19-hour)	Post-Prep (24 hours)
Bupropion	2.5	90.4%	94.9%	83.4%	70.5%	104.6%
Bupropion	250	96.5%	94.3%	90.3%	78.0%	100.4%
Hydroxy bupropion	10	99.8%		99.6%		100.2%
Hydroxy bupropion	750	98.8%		97.9%		95.8%
Erythro bupropion	2.5	97.5%		103.4%		101.7%
Erythro bupropion	100	98.4%		101.6%		99.1%
Threo bupropion	2.5	97.7%		95.1%		103.6%
Threo bupropion	200	99.9%		99.6%		97.9%

Figure 12: Plasma benchtop stability over 19 hours at 22°C



Bupropion was found to be stable for 1-hour in human plasma (i.e. time necessary for sample preparation). Bupropion was found to degrade significantly over a 19-hour time period. Degradation was observed to be concentration dependent, with the lower concentration of 2.5 ng/mL having the greatest loss of the parent compound. These observations were similar to Laizure.

Based on literature and DPQR laboratory observations it was determined that bupropion aqueous stock solutions (O'Byrne, 2010) should be prepared at pH below 5 and processed at a pH below 5 to ensure biostability (Chen, 2005, Laizure, 1985). In summary the stability of bupropion and its metabolites were found to be acceptable under stability conditions tested.

Recovery

Table 17: Recovery of bupropion and metabolites and internal standard from human plasma

Experiment	Plasma Conc. ng/mL	H-Bupropion	Bupropion	E-Bupropion	Br-Bupropion	T-Bupropion
1	5/20/50	62.1%	76.7%	75.7%		76.1%
2	"	67.0%	75.5%	75.7%	74.4%	72.3%
3	"	65.1%	78.1%	80.9%	81.3%	76.7%
4	"			79.1%	75.2%	79.5%
5	50/100/50	68.7%	80.1%	77.0%	78.0%	76.5%
1	"	63.6%	77.9%	77.3%		76.0%
4	"	64.4%	75.2%	74.5%	76.0%	75.3%
6	100/250/50	68.5%	79.1%	79.2%		75.9%
7	"	62.5%	76.8%	73.7%		75.5%
4	"	66.8%	74.3%	76.8%	75.3%	75.2%
8	200/400/50	69.9%	80.2%	79.9%		79.0%
7	"	65.1%	77.3%	76.6%		75.8%
4	"	65.6%	77.9%	76.1%	76.7%	76.7%
9	250/750/50	73.9%	80.2%	77.1%		76.6%
10	"	67.2%			81.5%	
7	"	65.9%	75.3%	73.7%		75.2%
4	"	68.1%	79.3%	77.0%	78.4%	77.0%
	Average	**67.4%**	**77.6%**	**77.5%**	**78.7%**	**76.3%**
	Standard Deviation	2.8%	2.1%	2.1%	2.5%	1.9%

The recovery of bupropion and its metabolites was determined from human plasma at concentrations over the analytical range. Experiments were conducted on 10 analysis days with compound addition and concentration staggered over the course of the recovery study. Recovery of bupropion, its metabolites and the internal standard was determined to be consistent, reproducible and correlative.

Incurred Recovery Samples

Nine patients were selected from each period for the incurred recovery study. For each patient, the C_{max} sample and an elimination phase sample were re-extracted as "incurred samples." Incurred sample data were compared to the original data by the formula

Variability(%) = ((repeat - original)/mean)*100%

with a value beyond ±20% indicating failure for the sample. Proposed rules on incurred sample variability allow for no more than 33% of samples to fail. In this study, no more than 11% of samples failed for bupropion or any one metabolite.

Table 18: Incurred Sample Reanalysis Results

	Original				Incurred				Variability(%) = ((repeat - original)/mean)*100%			
Sample	Bupropion	E Bupropion	T Bupropion	H Bupropion	Bupropion	E Bupropion	T Bupropion	H Bupropion	Bupropion	E Bupropion	T Bupropion	H Bupropion
Patient 1 Study A Time 6 hr	117.8	19.2	118.2	512.6	119.9	18.5	117.1	522.3	1.76%	-3.61%	-0.93%	1.86%
Patient 1 Study A Time 16 hr	27.4	27.3	109.0	559.5	28.0	26.5	108.5	599.3	2.15%	-3.09%	-0.42%	6.86%
Patient 3 Study B Time 2 hr	111.4	6.2	61.2	253.1	94.3	5.2	62.6	268.2	-16.65%	-17.11%	2.35%	5.77%
Patient 3 Study B Time 24 hr	20.8	11.9	70.7	563.7	19.9	12.2	78.4	632.9	-4.33%	2.02%	10.25%	11.58%
Patient 4R Study B Time 5 hr	114.8	24.9	124.2	456.2	108.1	21.9	117.2	405.5	-5.97%	-12.88%	-5.80%	-11.77%
Patient 4R Study B Time 12 hr	35.4	29.2	111.1	461.8	32.7	24.5	99.6	377.2	-7.97%	-17.48%	-10.93%	-20.15%
Patient 6 Study B Time 4 hr	117.2	30.4	236.9	228.5	109.7	30.9	246.4	234.5	-6.55%	1.79%	3.92%	2.59%
Patient 6 Study B Time 12 hr	32.5	31.8	230.9	301.9	32.3	35.3	245.4	341.1	-0.71%	10.32%	6.07%	12.18%
Patient 7 Study B Time 2 hr	112.2	9.7	84.1	221.0	94.9	8.6	77.7	201.7	-16.72%	-12.33%	-7.84%	-9.14%
Patient 7 Study B Time 24 hr	21.1	26.9	113.8	441.6	21.7	24.0	110.2	393.6	2.71%	-11.27%	-3.25%	-11.49%
Patient 8 Study A Time 5 hr	117.9	24.9	179.4	587.6	111.2	25.1	185.9	526.9	-5.86%	0.64%	3.55%	-10.89%
Patient 8 Study A Time 16 hr	21.8	22.8	140.6	488.8	20.6	22.9	153.5	501.9	-5.77%	0.50%	8.79%	2.64%
Patient 10 Study B Time 3 hr	156.4	20.0	116.7	359.2	136.4	16.4	106.6	306.7	-13.63%	-19.99%	-9.06%	-15.78%
Patient 10 Study B Time 16 hr	20.1	26.1	102.3	483.7	22.2	15.6	64.2	270.8	9.91%	-50.82% *	-45.80% *	-56.42% *
Patient 11 Study B Time 4 hr	80.3	12.4	53.2	214.9	18.3	22.2	93.8	399.6	-125.73% *	56.35% *	55.27% *	60.14% *
Patient 11 Study B Time 16 hr	22.3	15.7	55.4	240.5	74.4	13.4	58.0	239.6	107.80% *	-15.60%	4.59%	-0.36%
Patient 12R Study A Time 6 hr	112.8	34.7	195.2	722.6	92.0	31.2	194.4	625.6	-20.31%	-10.62%	-0.39%	-14.39%
Patient 12R Study A Time 16 hr	28.9	40.6	172.6	638.2	22.7	33.9	158.4	523.6	-23.94%	-17.84%	-8.55%	-19.74%
Patient 14 Study A Time 3 hr	49.8	12.7	84.8	85.6	47.2	13.7	90.4	99.7	-5.30%	7.74%	6.38%	15.22%
Patient 14 Study A Time 12 hr	29.6	17.2	98.0	113.4	27.2	16.9	97.5	137.2	-8.37%	-1.82%	-0.54%	18.93%
Patient 15 Study A Time 4 hr	229.2	26.3	218.5	163.4	198.1	23.5	206.5	154.3	-14.60%	-10.94%	-5.64%	-5.76%
Patient 15 Study A Time 24 hr	33.9	45.8	185.5	324.2	32.7	40.6	189.8	305.5	-3.56%	-12.07%	2.28%	-5.94%
Patient 16 Study B Time 4 hr	159.5	23.6	143.8	343.8	147.5	21.4	136.0	306.9	-7.80%	-9.41%	-5.63%	-11.33%
Patient 16 Study B Time 16 hr	39.8	30.9	125.1	546.2	33.8	26.3	111.6	465.3	-16.29%	-16.12%	-11.42%	-16.00%
Patient 17 Study A Time 5 hr	57.9	18.4	132.9	300.6	53.5	18.6	136.8	274.0	-7.79%	1.15%	2.91%	-9.26%
Patient 17 Study A Time 16 hr	24.5	20.8	121.7	254.4	22.1	20.4	118.1	269.5	-10.30%	-1.77%	-3.01%	5.77%
Patient 18 Study A Time 4 hr	65.1	12.6	50.3	377.4	60.5	12.2	50.1	383.7	-7.24%	-2.64%	-0.46%	1.64%
Patient 18 Study A Time 16 hr	19.5	15.2	54.9	322.1	18.5	14.8	53.7	368.4	-5.25%	-2.34%	-2.22%	13.41%
Patient 20 Study A Time 4 hr	124.9	13.5	82.2	329.3	117.1	13.0	83.7	298.0	-6.49%	-3.87%	1.83%	-9.96%
Patient 20 Study A Time 16 hr	20.8	13.8	52.6	321.0	18.1	12.8	50.5	259.8	-14.23%	-7.87%	-4.10%	-21.07%
Patient 21 Study B Time 4 hr	48.4	12.2	71.2	191.9	48.1	11.5	73.0	194.1	-0.68%	-5.27%	2.60%	1.14%
Patient 21 Study B Time 16 hr	23.7	24.2	109.2	256.9	23.5	22.7	112.0	266.9	-0.70%	-6.18%	2.53%	3.83%
Patient 23 Study A Time 5 hr	132.6	30.6	171.0	702.8	124.7	26.8	165.3	644.8	-6.16%	-13.15%	-3.42%	-8.61%
Patient 23 Study A Time 16 hr	22.5	28.8	114.6	647.7	19.8	26.6	105.6	646.3	-12.76%	-8.26%	-8.17%	-0.21%
Patient 10R Study B Time 5 hr	75.2	15.7	90.7	322.8	62.5	12.1	77.1	270.8	-18.42%	-25.77%	-16.15%	-17.49%
Patient 10R Study B Time 24 hr	38.7	29.6	112.1	557.8	34.8	26.2	112.9	527.6	-10.52%	-11.92%	0.78%	-5.57%

three samples (10-B-16, 11-B-4, and 11-B-16) show significant deviation, possibly due to an inadvertant shift in sample placement during processing

Sample Reanalysis (samples exceeded calibration curve)

During the analysis of patient samples data, several samples were determined to have concentrations higher than the validated calibration curve range for one or more of the analyzed analytes. These samples were extracted a second time after dilution with blank plasma, and reanalyzed. The results from the original and second extractions of these samples are displayed in Table 19 for comparison.

Table 19: Reanalysis of samples that exceeded calibration curve

	H-Bupropion		Bupropion		E-Bupropion		T-Bupropion	
Sample	1st run	2nd run	1st run	2nd run	1st run	2nd run	1st run	2nd run
Patient 06, Study A, Time 12	264.7	263.4	37.7	40.6	29.6	30.5	205.2	207.9
Patient 06, Study A, Time 16	287.8	281.5	30.9	32.8	31.9	32.6	216.5	219.4
Patient 06, Study B, Time 4	228.5	219.4	117.2	124.7	30.4	29.9	237.3	236.9
Patient 06, Study B, Time 5	253.4	271.8	108.4	120.5	35.4	36.9	268.7	286.9
Patient 06, Study B, Time 6	272.8	284.8	86.4	97.7	34.0	35.2	255.2	272.1
Patient 06, Study B, Time 8	296.7	300.5	57.1	67.0	34.8	36.7	249.3	258.2
Patient 06, Study B, Time 12	301.9	314.3	32.5	38.8	31.8	34.1	219.0	230.9
Patient 06, Study B, Time 16	298.9	295.6	16.4	17.8	32.4	32.9	209.6	212.3
Patient 07, Study A, Time 12	714.8	715.5	114.1	109.2	41.0	38.9	232.0	233.8
Patient 07, Study A, Time 16	669.9	619.2	62.4	54.0	38.2	36.2	204.2	190.5
Patient 09, Study A, Time 24	433.0	424.7	15.5	11.7	20.9	20.5	75.3	69.1
Patient 09, Study A, Time 36	297.0	297.5	9.7	7.7	13.9	13.9	46.3	46.8
Patient 09, Study A, Time 48	233.2	248.8	8.5	6.5	12.8	12.9	43.9	43.5
Patient 09, Study A, Time 60	162.0	159.6	6.2	4.8	8.5	8.4	31.4	29.6
Patient 15, Study A, Time 4	163.4	164.4	229.2	243.4	26.3	24.8	211.9	218.5
Patient 15, Study A, Time 5	216.3	207.9	144.5	144.2	28.3	25.2	207.5	199.8
Patient 15, Study A, Time 6	260.3	252.4	143.6	146.9	31.5	29.6	222.0	223.4
Patient 15, Study A, Time 8	319.1	305.0	131.5	131.2	42.7	39.3	266.5	270.6
Patient 15, Study A, Time 12	376.4	351.1	95.0	94.8	51.6	46.6	280.6	281.8
Patient 15, Study A, Time 16	375.0	364.4	67.4	67.1	54.0	49.8	258.2	261.3
Patient 15, Study B, Time 8	209.5	230.9	79.8	84.1	36.2	31.2	201.8	196.2
Patient 15, Study B, Time 12	210.9	239.2	59.0	62.0	41.7	36.2	219.7	206.5
Patient 15, Study B, Time 16	197.6	217.2	41.0	41.7	42.6	37.5	203.1	193.9
Patient 20, Study B, Time 6	455.6	423.3	284.3	278.4	22.6	22.5	170.2	164.2
Patient 23, Study A, Time 8	756.0	735.9	75.9	74.9	32.9	32.0	159.5	157.6
Patient 23, Study A, Time 12	777.2	785.0	49.8	48.9	33.8	32.1	143.3	135.6
Patient 23, Study A, Time 24	774.7	742.2	16.0	15.3	28.4	26.6	97.8	90.9

Dilution integrity was established at the 2-fold (1;2 dilution) and 4-fold levels (1:4 dilution) in human plasma.

Database Quality Assurance

An MS Excel scientifically randomized dataset was created to evaluate 1,056 of 7,512 sample data points for the bupropion clinical study. Three bupropion team members were selected to conduct the quality assurance evaluation of the bupropion database. The study coordinator who created the bupropion database for randomized sampling did not participate in the direct evaluation of the bupropion database quality assurance study.

Scientist 1 was assigned patients: 1, 4R, 6, 10, 11, 13, 15, 16, 18, 21, 23, 24 (12 patients)
Scientist 2 was assigned patients: 2, 5, 8, 9, 14, 17, 20, 22 (8 patients)
Scientist 3 was assigned patients: 3, 7, 12R, 19 (4 patients)

The protocol was as follows:
Step 1: Check out assigned bupropion patient file.
Step 2: Identify raw data on Waters UPLC-1 computer for the following: system suitability standards, quality controls, calibration standards and patient's data from phase A and phase B.
Step 3: Cross check raw data from instrument and hard copy from patient file.
Step 4: Cross check hard copy data and data in the bupropion database for system suitability, quality controls, calibration standards and patients data from phase A and phase B.
Step: 5: Send an individual file report to the study coordinator. The coordinator or his representatives will compile the evaluation data and prepare a statistical assessment of the error rate in the bupropion database.

Summary

Quality of data entry was verified by independent checking of approximately 15% of the sample analysis data points that were manually transcribed from the analysis system to the computational software. Zero errors were found for the approximately 1056 of 7,512 sample analysis data points that were evaluated in the bupropion BE database.

PHARMACOKINETICS ANALYSIS

C_{max} and T_{max} were observed values. The other PK parameters and 90% confidence intervals (CIs) were calculated using SAS software. The following tables display the ratio between test and reference products and 90% CI.

Table 20a: PK Ratios for Bupropion (Test / Reference)

Summary of Statistical Analysis (n=24) for Bupropion Ratios (Test/Reference)		
Parameter	Point Estimate	90% Confidence Interval
AUC_t	0.86	76.71-95.82
AUC_{inf}	0.87	78.88-96.97
C_{max}	0.75	65.24-86.81

Table 20b: PK Ratios for Hydroxy Bupropion (Test / Reference)

Summary of Statistical Analysis (n=24) for Hydroxy Bupropion Ratios (Test/Reference)		
Parameter	Point Estimate	90% Confidence Interval
AUC_t	0.88	81.04-96.07
AUC_{inf}	0.89	80.11-98.09
C_{max}	0.87	76.46-89.73

Table 20c: PK Ratios for Erythro Bupropion (Test / Reference)

Summary of Statistical Analysis (n=24) for Erythro Bupropion Ratios (Test/Reference)		
Parameter	Point Estimate	90% Confidence Interval
AUC_t	0.95	85.84-104.81
AUC_{inf}	1.02	90.50-115.11
C_{max}	0.91	83.23-99.76

Table 20d: PK Ratios for Threo Bupropion (Test / Reference)

Summary of Statistical Analysis (n=24) for Threo Bupropion Ratios (Test/Reference)		
Parameter	Point Estimate	90% Confidence Interval
AUC_t	0.96	87.71-105.70
AUC_{inf}	1.02	91.07-114.94
C_{max}	0.82	73.82-91.43

Table 21a: PK summary for bupropion

Bupropion						
Parameters	**Units**	**Test**		**Reference**		**T/R**
		Mean	%CV	Mean	%CV	
AUC_t	ng/mL x hr	1180.228	31.16	1400.867	38.35	0.84
AUC_{inf}	ng hr/mL x hr	1316.268	32.08	1527.465	38.28	0.86
C_{max}	ng/mL	86.611	32.28	120.511	47.65	0.72
T_{max}	Hr	4.000	38.14	5.000	23.37	0.80
K_e	hr^{-1}	0.041	61.83	0.040	52.65	1.04
$T_{1/2}$	Hr	23.689	63.99	20.980	36.04	1.13

Table 21b: PK Summary for Hydroxy Bupropion

Hydroxy Bupropion						
Parameters	**Units**	**Test**		**Reference**		**T/R**
		Mean	%CV	Mean	%CV	
AUC_t	ng/mL x hr	18861.83	39.91	20636.35	31.68	0.91
AUC_{inf}	ng hr/mL x hr	24772.70	44.79	26931.32	39.52	0.92
C_{max}	ng/mL	432.241	37.24	505.023	29.84	0.86
T_{max}	Hr	16.000	49.42	12.000	46.13	1.33
K_e	hr^{-1}	0.026	33.32	0.027	30.54	0.97
$T_{1/2}$	Hr	29.896	36.28	30.964	64.91	0.97

Table 21c: PK Summary for Erythro Bupropion

Etythro Bupropion						
Parameters	**Units**	**Test**		**Reference**		**T/R**
		Mean	%CV	Mean	%CV	
AUC_t	ng/mL x hr	1192.096	35.63	1239.500	32.90	0.96
AUC_{inf}	ng hr/mL x hr	1791.743	41.72	1746.011	42.95	1.03
C_{max}	ng/mL	25.451	28.50	28.251	32.56	0.90
T_{max}	Hr	16.000	53.81	12.000	43.22	1.33
K_e	hr^{-1}	0.020	37.57	0.022	32.23	0.91
$T_{1/2}$	Hr	42.101	65.74	34.441	32.27	1.22

Table 21d: PK Summary for Threo Bupropion

Threo Bupropion						
Parameters	**Units**	**Test**		**Reference**		**T/R**
		Mean	%CV	Mean	%CV	
AUC_t	ng/mL x hr	5624.606	42.93	5757.970	39.82	0.98
AUC_{inf}	ng hr/mL x hr	9788.317	50.90	9560.290	47.82	1.02
C_{max}	ng/mL	127.505	36.11	156.370	39.97	0.82
T_{max}	Hr	8.000	51.32	6.000	40.25	1.33
K_e	hr^{-1}	0.014	41.91	0.017	50.40	0.87
$T_{1/2}$	Hr	57.315	49.43	55.139	68.94	1.04

Figure 13a: Time course for bupropion

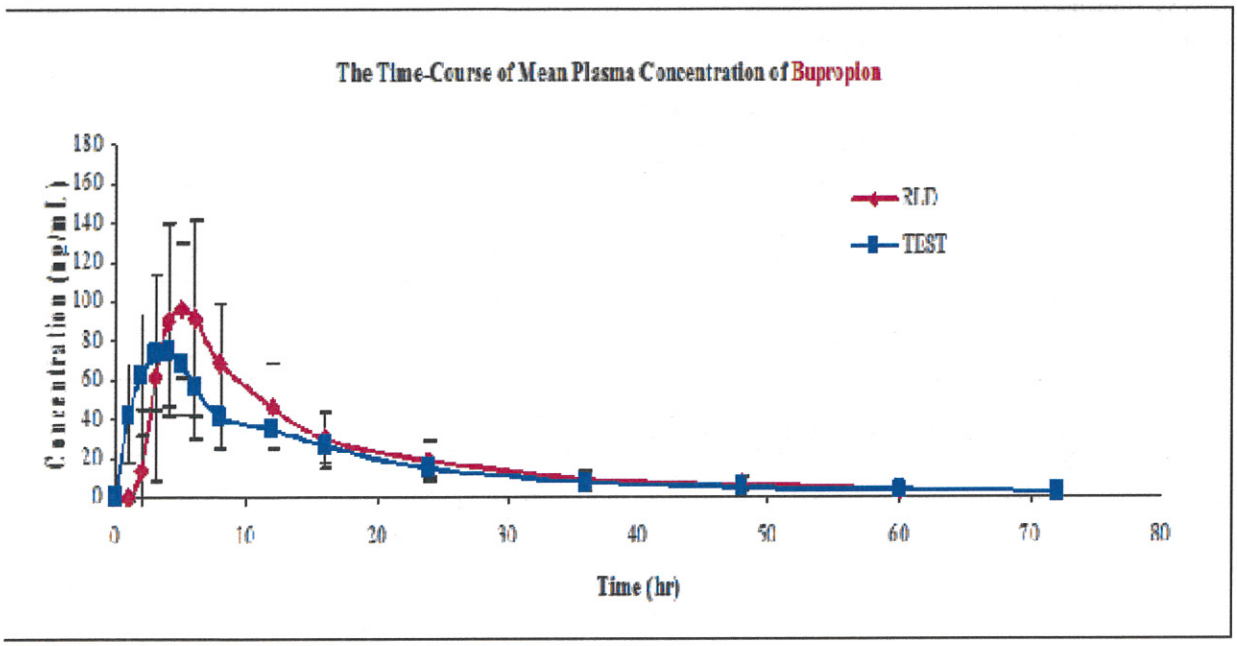


Figure 13b: Time course for erythro bupropion

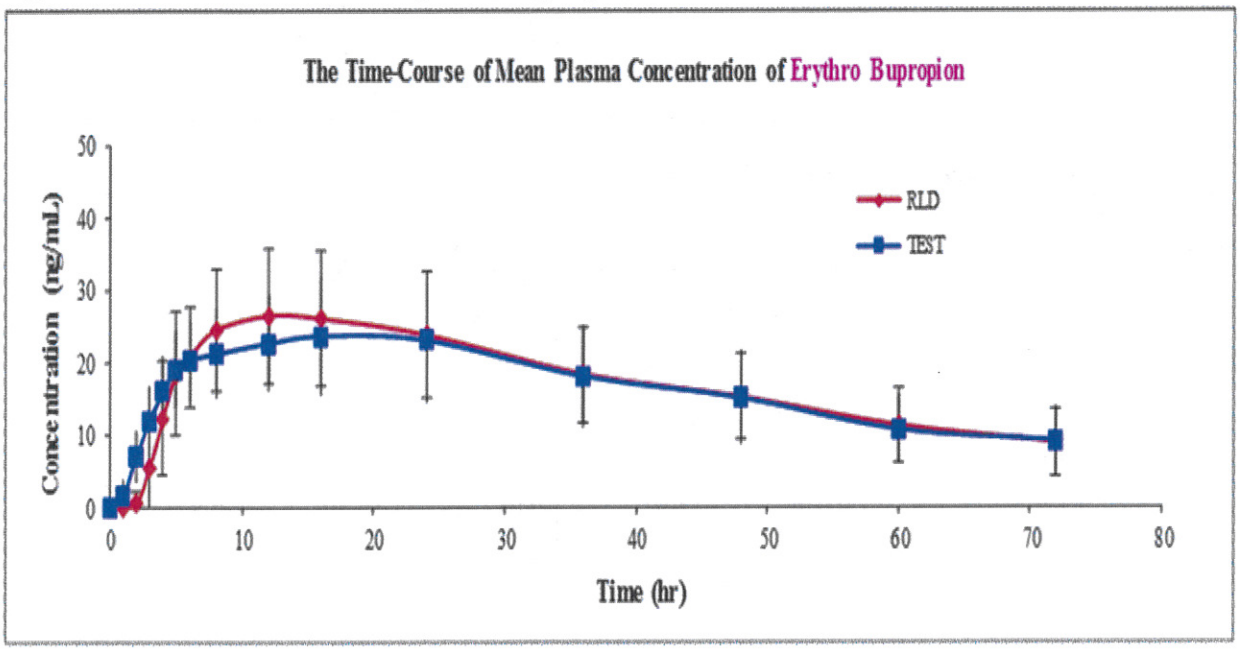


Figure 13c: Time course for threo bupropion

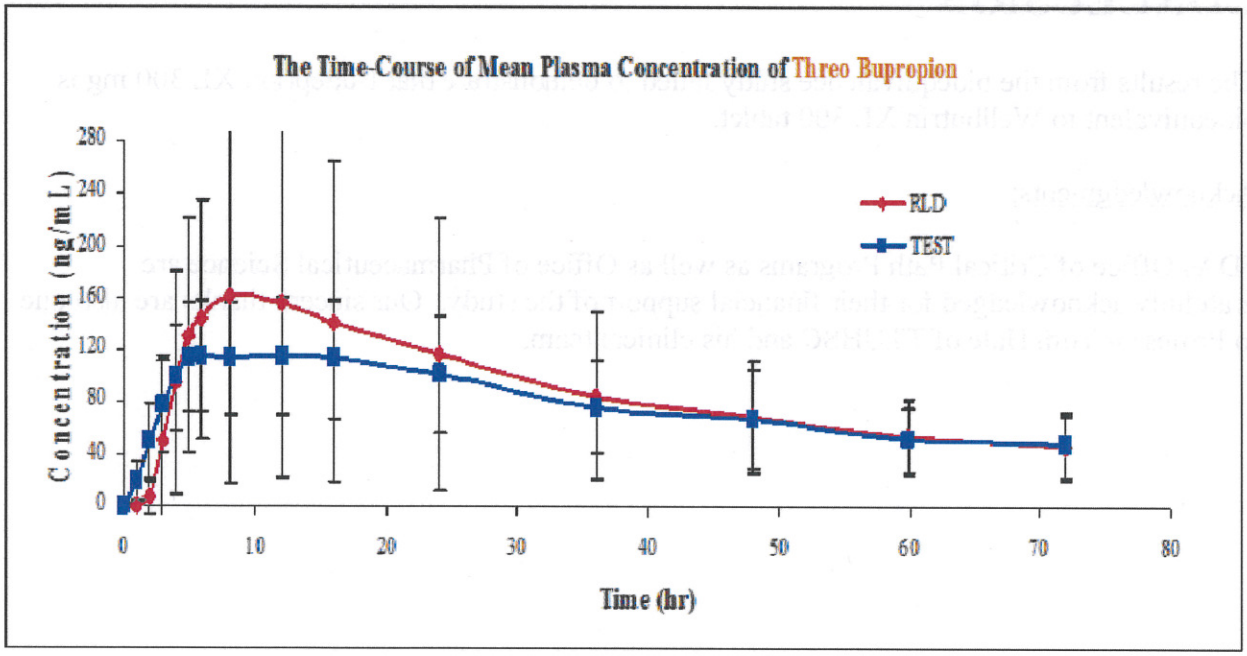
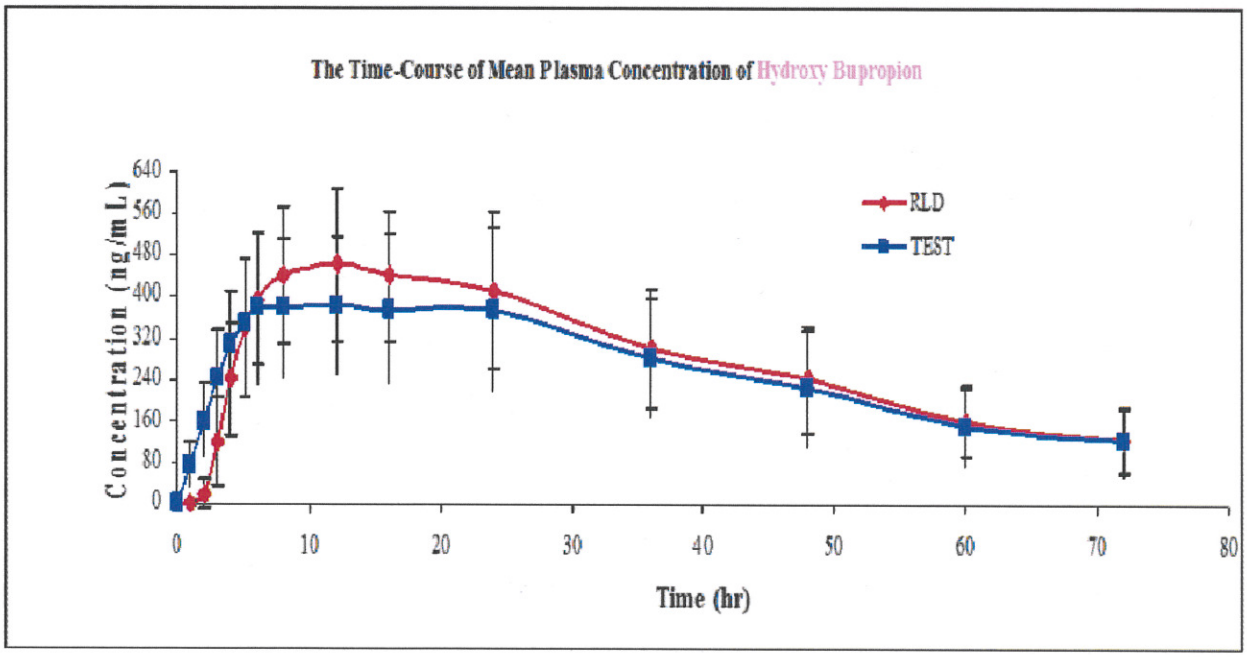


Figure 13d: Time course for hydroxy bupropion



CONCLUSION

The results from the bioequivalence study failed to demonstrate that Budeprion XL 300 mg is bioequivalent to Wellbutrin XL 300 tablet.

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