IJDDR
International Journal of Drug Development & Research

Liquid Chromatography Tandem mass spectrometry method for Quantification of Buproprion and Hydroxy Buproprion in Human Plasma and its application to Bioequivalence Study

Peeyush Jain[1], Nishant Paliwal[1], Naveen Dubey[1], Sandeep Sharma[1] and Bhawani Singh[2]

[1] Jubilant Clinsys Ltd, C-46, Sector-62, NOIDA (UP)-201 307, India
[2] Department of Chemistry, Banasthali Vidyapith (Rajasthan)-304 022, India

Abstract

A rapid, specific and robust assay based on solid phase extraction and liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI MS-MS) has been developed and validated for the quantitative analysis of Buproprion (a drug used for smoking cessation) in human plasma using Buproprion D9 as internal standard (ISTD). The precursors to product ion transitions of m/z 240.20/183.90 and m/z 256.10/237.90 were used to measure the analyte (Buproprion and hydroxy Buproprion) and the precursor to product ion transition of m/z 249.20/131.00 was used to measure the ISTD. The method was validated over a concentration range of 1.00ng mL^{-1} to 304.65ng mL^{-1} for Bupropion and 3.00ng mL^{-1} to 801.78ng mL^{-1} for hydroxy Bupropion. The method was validated over the various parameters like selectivity, matrix effect, sensitivity, linearity, precision, accuracy, stabilities (bench-top stability, standard stock solution stability in refrigerator and at room temperature, stock dilution stability, auto-sampler stability, freeze thaw stability, long-term stability at -65°C ± 10°C & -22°C ± 5°C, reagent stability, dry-extract stability, wet-extract stability and blood stability), effect of potentially interfering drugs, dilution integrity, recovery, reinjection reproducibility, ruggedness, extended batch verification, ion-suppression through infusion and inter-conversion check *etc*. The mean percent recovery of Bupropion was found 58.443% with a precision of 1.01% whereas the mean percent recoveries of hydroxy Bupropion and Bupropion D9 were found 62.327% and 66.513% with a precision of 2.99% and 3.91% respectively. The RSD percent of intra-day and inter-day assay was ≤15%. The application of this assay was demonstrated in a bioequivalence study and it was found suitable for a study of sample size as big as sixty enrolled volunteers.

*Corresponding author, Mailing address:
Peeyush Jain
E-Mail: peeyush_jain108@yahoo.com

How to Cite this Paper:

Peeyush Jain, Nishant Paliwal, Naveen Dubey, Sandeep Sharma and Bhawani Singh "Liquid Chromatography Tandem mass spectrometry method for Quantification of Buproprion and Hydroxy Buproprion in Human Plasma and its application to Bioequivalence Study" Int. J. Drug Dev. & Res., April-June 2012, 4(2): 381-392

INTRODUCTION

Cigarette smoking is considered as a leisure habit that has imposed substantial health and financial

costs on individuals and society. Smoking is a principal cause of cancer that may affect lungs, esophagus, larynx, mouth, throat, kidney, bladder, pancreas, stomach or cervix. Dependence over nicotine and the associated withdrawal symptoms in persons consuming cigarette have been presented as significant limitations in the treatment, until recently, the options available were in the form of nicotine replacement therapy (NRT). Bupropion has been emerged as the first non-nicotine based drug for smoking cessation. Bupropion hydrochloride [(+/-)-2-tert-butylamino-3'-chloropropiophenone.HCl] is a nonselective inhibitor of the dopamine and norepinephrine transporter and it also inhibits neuronal nicotinic acetylcholine receptors[1]. Bupropion was developed as a drug of choice for the treatment of major depressive disorder, but it has been shown potential in attenuating the expression of nicotine withdrawal symptoms. During smoking withdrawal, Bupropion attenuate symptoms by mimicking nicotinic effects through the inhibition of dopamine reuptake into neuronal synaptic vesicles. Bupropion is metabolized to Hydroxybupropion by CYP2B6, an isoenzyme of the cytochrome P450 system. Bupropion bears highly variable metabolism among different individuals as a result of which the concentration levels of both drug and metabolite needs to be monitored during the pharmacokinetic studies[2]. Effective assessment of the pharmacokinetic profile of the drug in body is possible through the development and validation of a sensitive and robust method for the estimation of both Bupropion and Hydroxybuprprion in human plasma.

Few analytical methods have been reported for the determination of Buproprion that include HPLC with UV detection and HPLC coupled with MS/MS for better sensitivity[3-7]. Recently, JM Parekh *et al.* has reported a simultaneous method for determination of Buproprion and its metabolites in human plasma and

its use in bioequivalence study in which the method was developed using venalafexine as an internal standard. Buprprion is structurally dissimilar to venlafaxine so both of them use to show dissimilarity in extraction and elution during the analysis. This disparate behavior of the analyte and internal standard can affect the outcome of the results in a longer bioequivalence study as the sudden dip or rise of the area ratio which can change the regression coefficient of the calibration curves across the study (%RSD of ≤ 15% for the regression coefficient is recommended)[8]. A fewer LC-MS/MS methods have been reported for the determination of Buproprion and Hydroxybuproprion in rat and human plasma, however, the ruggedness of these methods were not demonstrated for a longer bioequivalence study. KK Loboz *et al.* has reported a HPLC based method for the determination of Buproprion and its major metabolites in human plasma in which the method was reported to have lower sensitivity and longer run time that compromises its use in pharmacokinetic studies. In the present work, a sensitive, fast and robust method has been developed and validated for the determination of Buproprion and Hydroxybuproprion in human plasma using Buproprion D9 as an internal standard. The use of method has also demonstrated to conduct bioequivalence study more effectively.

EXPERIMENTAL

Materials and Method

Buproprion (>99.9% *w/w* basis), Hydroxybuproprion (>97.8% *w/w* basis) and Buproprion D9 (>99.4% *w/w* assay by HPLC), were obtained from Clearsynth Lab Limited, India (Fig. 1). HPLC-grade methanol purchased from SD Fine Chem. Ltd. (Mumbai, India). HPLC-grade acetonitrile purchased from JT Baker (Phillipsburg, USA). Ammonium acetate and formic acid purchased from Fluka (Fluka Chemie, GmbH, Germany). Milli-Q water 8.2m Ω(milliohm) and TOC ≤ 50 ppb (parts

per billion)] from Milli-Q system (Millipore SAS, Molsheim, France) was used. Phenomenex 100 x 4.6 mm, 5µm column purchased from Phenomenex, USA. All other reagents and chemicals used for these studies were of HPLC grade unless specified.

1 (a)

1 (b)

1 (c)

Fig. 1: Product ion spectra of (1a) Bupropion(1b) Hydroxybuproprion and 1(c) Buproprion D9

Instrumentation

LC-MS/MS analysis was performed using API 3000 triple quadrupole instrument (Applied Biosystems MDS SCIEX, Toronto, Canada) coupled with Shimadzu HPLC system (Shimadzu SIL HTC, USA) in multiple reaction monitoring (MRM) mode. A turbo electronspray interface in positive ionization mode was used for ionization. Data processing was performed on Analyst software version 1.4.2 (Applied Biosystems MDS SCIEX, Toronto, Canada).

## Standard and Quality Control Sample Preparation

Primary stock solution of Buproprion for preparation of calibration standard and quality control (QC) samples were prepared separately. The primary stock solution of Buproprion (1.0mg mL^{-1}), Hydroxybuproprion (1.0mg mL^{-1}) and Buproprion D9 (100µg mL^{-1}) were prepared in methanol. The stock solution of internal standard was diluted to concentration of (approximately 1500ng mL^{-1}) using diluent solution (Methanol: Milli-Q Water: 50:50, *v/v*) before being used for sample processing. Aqueous dilution for spiking were prepared by serially diluting the primary stock solution of Buproprion and Hydroxybuproprion in water:methanol (50:50, *v/v*). Spiking of aqueous dilutions in human plasma was done to give eight-point calibration curve (1.00 to 304.65ng mL^{-1}) for Bupropion and (3.00ng mL^{-1} to 801.78ng mL^{-1}) for Hydroxybupropion. In a similar way, spiking of aqueous quality control dilutions was done in human plasma to prepare the quality control samples consisting of Bupropion concentrations of 1.00ng mL^{-1} (LLOQ QC), 2.78ng mL^{-1} (LQC), 148.79ng mL^{-1} (MQC) and 260.15ng mL^{-1} (HQC) and Hydroxybupropion 3.00ng mL^{-1} (LLOQ QC), 8.15ng mL^{-1} (LQC), 407.30ng mL^{-1} (MQC) and 683.01ng mL^{-1} (HQC). Primary stock solutions were kept at 2-8°C when not in use. Spiked calibration standards and QC samples were stored below −65°C.
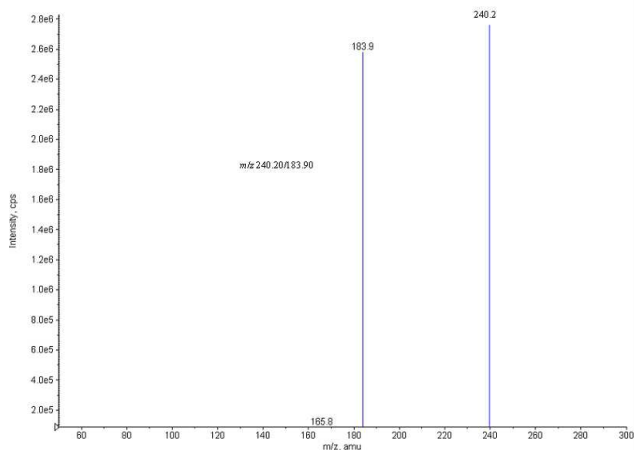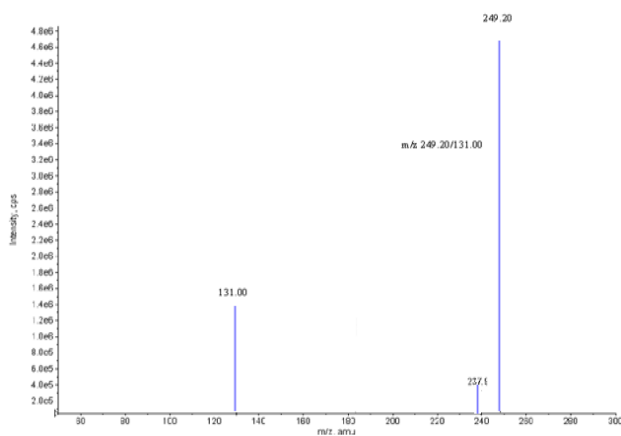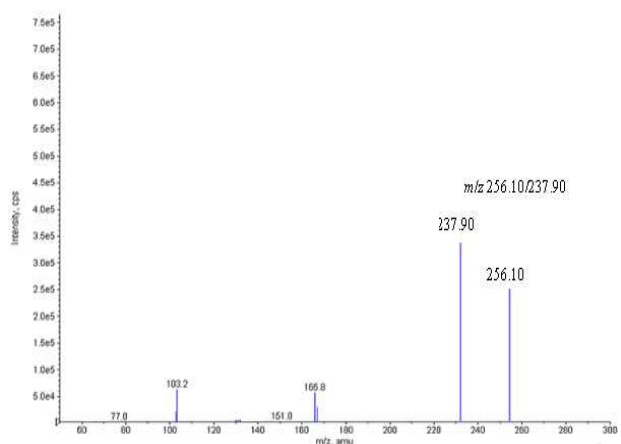
Sample Preparation

Sample preparation involved aliquoting of 200µl of plasma into ria vials and addition of 50µl internal standard dilution (Bupropion D9 approx 1500ng mL^{-1}) and 200µL of 1% formic acid solution to it followed by vortexing for a small time. This was followed by conditioning of the HLB cartridge (30 mg/1cc) with 1.0 ml methanol followed by 1.0 ml of Milli-Q water. The conditioned cartridges were then loaded with prepared sample. This was followed by washing the cartridges with 1 ml of buffer solution B. The cartridges were than dried for approximately 3 minutes. The dried cartridges were eluted of the sample with 1.0 ml of methanol into glass elution tubes. The eluate was evaporated to dryness at 40°C & at constant pressure in nitrogen evaporator followed by reconstitution of the dried samples in 300µl of mobile phase and transfer to HPLC vials for analysis.

Chromatographic and Mass Spectrometric Conditions

The analytes were chromatographically separated using reversed-phase high-performance liquid chromatography (HPLC) with isocratic elution. The mobile phase consisted of (Acetonitrile: 2mM Ammonium acetate in Milli-Q Water: 90:10, *v/v*) which was used at a flow rate of 1.0 mL min-^{1}. The chromatographic separation was performed using Phenomenex 100 x 4.6 mm, 5µm column. For all analyses, 5µL of extracted sample was injected. The total run time of 3.0 min was found suitable for taking apart the analyte and metabolite over the bonded phase with no late eluting peaks.

The mass spectrometer was operated in the electrospray ionization mode with positive ion detection to monitor the ions with *m/z* 240.20/183.90 for Buproprion, *m/z* 256.10/237.90 for Hydroxybuproprion and *m/z* 249.20/131.00 for Buproprion D9. For Buproprion and Buproprion D9, the source parameters optimized were nebulizer gas (NEB): 10psi, ion spray voltage (IS): 5000 V, source temperature (TEM): 350°C, collision gas (CAD): 4psi, curtain gas (CUR): 10psi, while the declustering potential (DP), collision energy (CE) and cell exit potential (CXP) applied were 30, 20 and 11 V for Buproprion and Buproprion D9 whereas they were 24, 18 and 11 for Hydroxybuproprion. The focusing potential was optimized at 152.00, 145.00 and 100.00 V for Buproprion, Hydroxybuproprion and Buproprion D9.

Data processing and Regression

The MRM chromatographic peaks were integrated using Analyst software version 1.4.2 after which peak area ratios of Buproprion to Buproprion D9 and Hydroxybuproprion to Buproprion D9 were plotted versus concentration and a linear curve fit, weighted by $1/x^2$ (where x = concentration) was used to produce the regression line.

Bioanalytical Method Validation

As a part of method validation, stabilities (bench top stability, standard stock solution stability in refrigerator and at room temperature, stock dilution stability, auto-sampler stability, freeze thaw stability, long term stability at -65°C ± 10°C & at - 22°C ± 5°C, reagent stability, dry extract stability, wet extract stability in refrigerator and on bench top, blood stability) were validated. The stock solution stability was evaluated at room temperature and for analytes (Bupropion and Hydroxy Bupropion) and internal standard (Bupropion D9) by the preparation of two aqueous mixtures one from the stability standard stock solution (kept on the bench at room temperature/in the refrigerator at 2-8°C) and the other from fresh standard stock solution (comparison stock) evaluated at 2-8°C. However, the stock dilution stability was evaluated at 2-8°C for analytes (Bupropion and Hydroxy Bupropion) and internal standard (Bupropion D9) by the preparation of two aqueous mixtures one from the stability stock

dilution solution (kept in the refrigerator at 2-8°C) and the other from fresh standard stock solution (comparison stock). An analysis of six replicates of aqueous mixture from stability stock and comparison stock was carried out to evaluate the stability. The percentage change of the mean response of the stability to the comparison stock and aqueous mixtures gives an estimate of the stability. To assess the auto-sampler stability of Bupropion and Hydroxybupropion, quality control samples of a pre-processed were stored into the auto-sampler at 5°C for the stability period. These samples were than quantified against freshly spiked calibration curve standards along with freshly spiked quality control samples. The stability of analyte in human plasma stored at room temperature (bench-top stability) was determined by processing bench top stability quality control samples and freshly spiked quality control samples and quantifying them against the freshly spiked calibration curve standards. The freeze-thaw stability was conducted by comparing the stability samples that had been frozen and thawed five times along with freshly spiked quality control samples against freshly prepared standards. The long-term stability was conducted by analyzing low and high quality control samples stored below -65°C and -22°C for 297 days along with freshly spiked quality control samples with freshly prepared calibration standards. Wet extract bench top and wet extract refrigerator stability of Bupropion and Hydroxybupropion was determined by processing and reconstituting six set of low and high quality control samples in mobile phase, keeping them on bench and in refrigerator (2-8°C) respectively and quantifying against freshly spiked calibration curve along with freshly spiked set of quality control samples. However the dry extract stability of Bupropion and Hydroxybupropion was determined by processing six set of low and high quality control samples, keeping them in refrigerator (-17 to -27 °C) respectively and quantifying against

freshly spiked calibration curve along with freshly spiked set of quality control samples.

RESULTS AND DISCUSSION
Method Development and Optimization

The scanning and acquisition of the parent and the product ions for Buproprion and Hydroxybuproprion was carried out by continuous infusion of the dilution of both analytes at appropriate concentration through a pump and sorting out appropriate polarity and ions from the finalized polarity mode. Afterwards optimization of mass spectrometric condition for each compound was carried out by continuous infusion and adjustment of the compound dependent parameters as declustering potential (DP), focusing potential (FP) and entrance potential (EP). Parameters such as DP, FP and EP were ramped to provide best signal to noise level for the parent ions. Afterwards optimization was carried out for the product ions to trace out the best combination of parameters as collision energy (CE), collision associated dissociation (CAD), cell exit potential (CXP). Afterwards source parameters as curtain gas, nebulizer gas, temperature were optimized by flow injection analysis was using a union in place of column. Then chromatographic conditions were optimized to look for sensitivity, peak shape and chromatographic run time. The selection of mobile phase was done taking into account symmetric peaks with shorter run time that further leads to low consumption of mobile phase altogether making the method cost effective. Results derived from several combinations showed that Acetonitrile: 2mM Ammonium acetate in Milli-Q Water: 90:10, *v/v*) serves the desired purpose with utmost effectiveness. An increase in organic content in the mobile phase aids in creating better orthogonal spraying condition within the ionization source. Addition of buffer as the aqueous component of the mobile phase helps in adjusting the pH of the mobile phase within the range of the *pKa* of the analytes.

Phenomenex 100 x 4.6 mm, 5µm column was found to be most suitable for analysis from an array of different varieties available as its bonded phase offers high percentage of hydrophobic retention and methylene selectivity. The use of proper internal standard was done to eliminate the quantitative bias caused by matrix effect and instrumental variation. Buproprion D9 was selected which has similar ionization condition, appropriate retention time and recovery compared to Buproprion leading to better tracking of analyte during the course of experiment.

### Assay Performance and Validation
### Selectivity and Sensitivity

Selectivity was the ability of the analytical method to differentiate and quantify the analyte in the presence of other expected components in the sample. The solid phase extraction procedure provides advance sample cleanup and gave very good sensitivity for the analysis of Buproprion, Hydroxybuproprion in human plasma. The selectivity was carried out in six normal plasma lots along with the six lots each of haemolysed and lipemic plasma. No interference of endogenous matrix/impurities was found at the retention time of the analyte and internal standard in normal haemolysed and lipemic plasma. Representative chromatograms of extracted blank human plasma (Fig. 2a) and blank human plasma fortified with ISTD (Fig. 2b) demonstrated the selectivity of the method. Sensitivity was determined by analyzing six replicates of blank human plasma spiked with the analyte at the lowest level of the calibration curve (1.00ng mL-1) for Buproprion and Hydroxybuproprion (3.00ng mL^{-1}). The representative chromatogram for the LLOQ (1.00 and 3.00ng mL^{-1}) showing sensitivity was depicted in Fig. 2c.
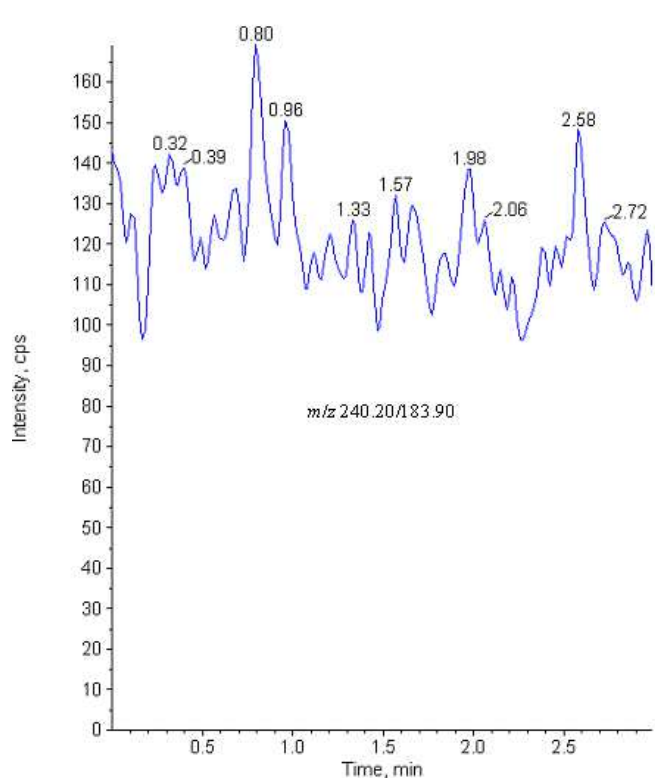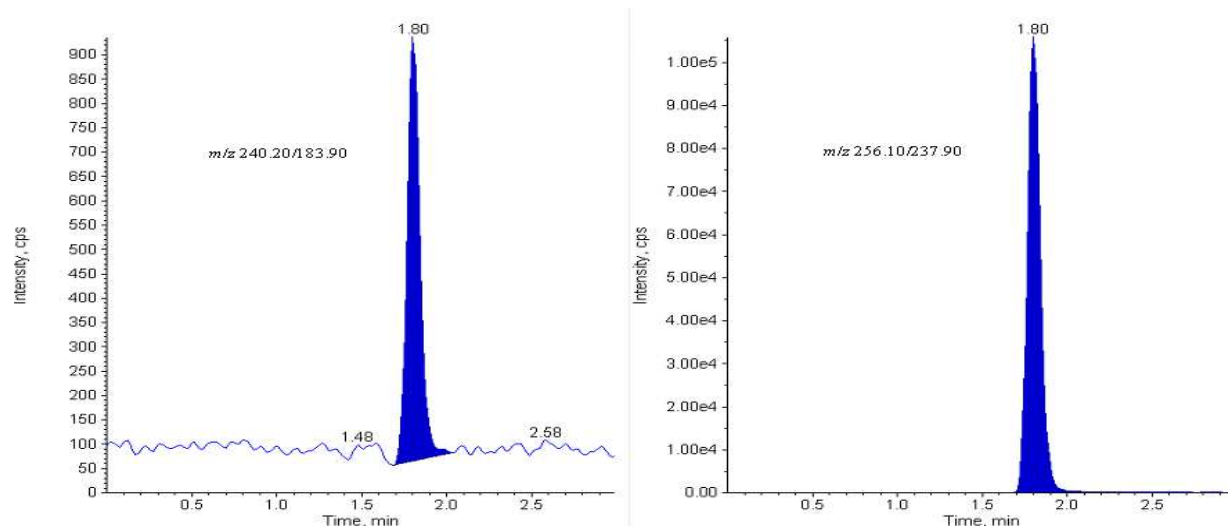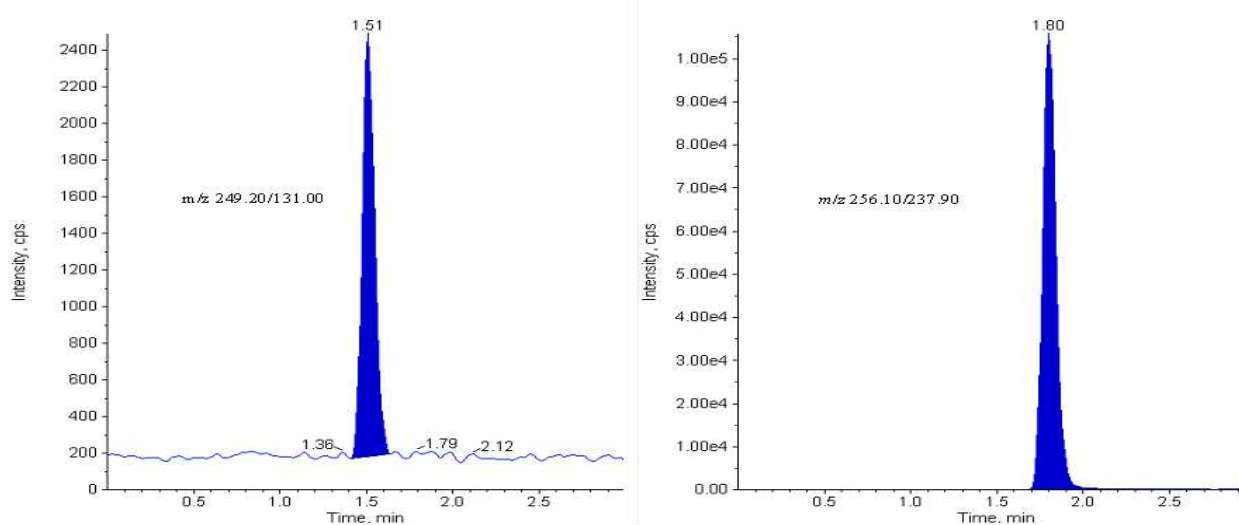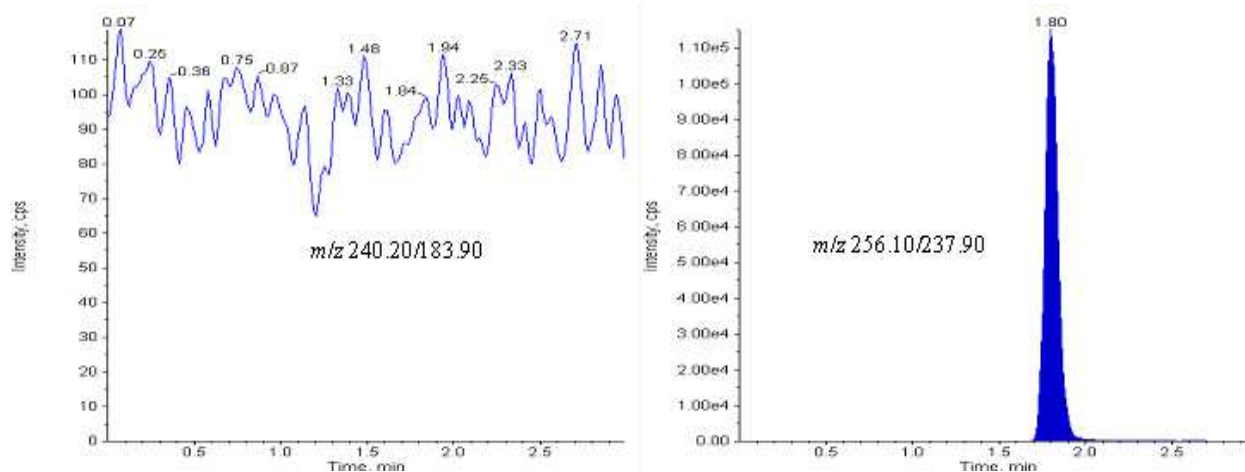

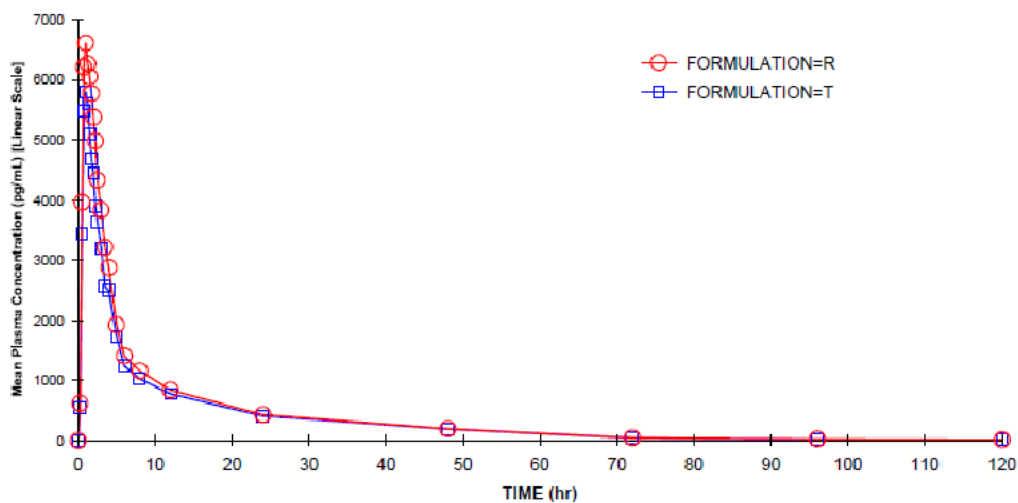**2(a1)**
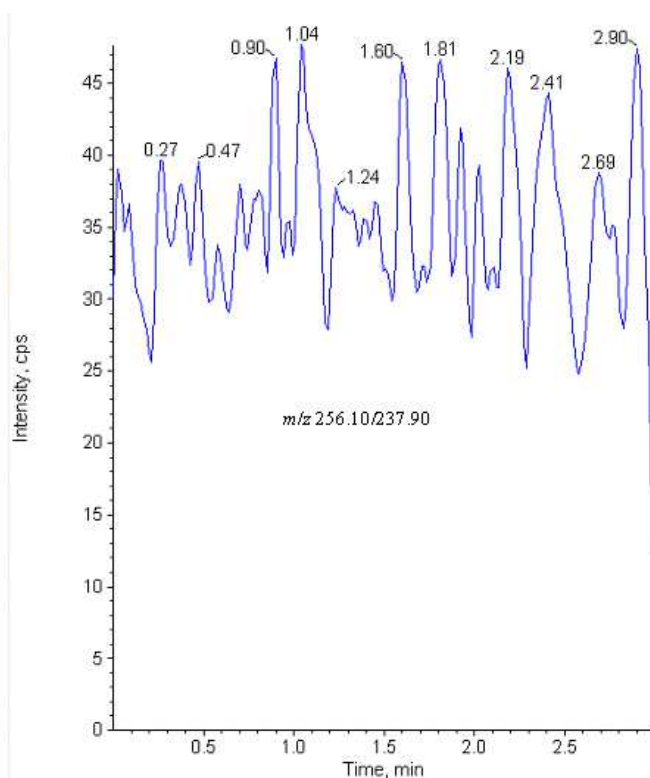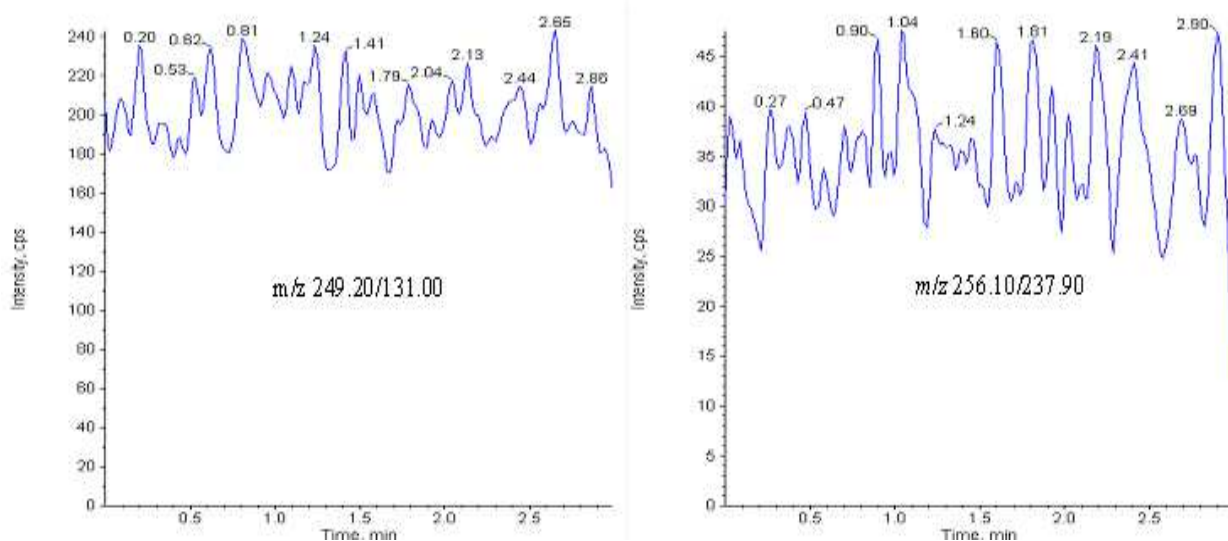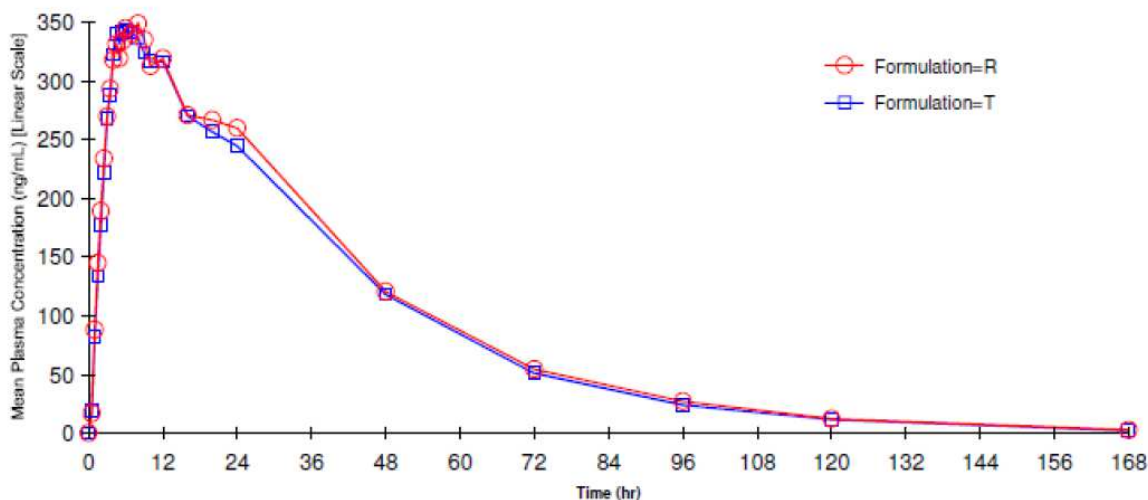
2(a2)

2(b1)

2(b2)

2(c1)

(2c2)

Fig. 2: MRM chromatogram of (2a1 and 2a2) blank plasma (2b1 and 2b2) blank spiked with Buproprion and Hydroxybuproprion at LLOQ and (2c1 and 2c2) blank spiked with ISTD.

3(a)

3(b)

Fig.3: Mean Plasma concentration-time profile for 3(a) Buproprion and 3(b) Hydroxybuproprion

Linearity

Calibration curves were linear over the concentration range 1.00 to 304.65ng mL^{-1} for Buproprion and 3.00 to 801.78ng mL^{-1} Hydroxybuproprion. Each batch of spiked plasma samples evaluated for linearity, precision and accuracy included one complete set of calibration curve standards and six replicates of quality control samples at LOQQC, LQC, MQC and HQC levels. The best linear fit and least square residuals for the calibration curve were achieved with a 1/x2 weighing factor, giving a mean linear equation for the calibration curve of y = (0.0082± 0.00013) x + (0.0003± 0.00050) where y was the peak area ratio of analyte to the ISTD and x was the concentration of analyte. The correlation coefficient(r) for Buproprion was above 0.999.

Precision and Accuracy

The linearity, precision and accuracy evaluations were performed on three batches of spiked plasma samples. The intraday precision and accuracy were calculated after repeated analysis in three analytical runs. The intra and inter-batch accuracy was determined by calculating percentage bias of quality control sample from the theoretical concentration. The within and between-day precision was determined in terms of relative standard deviation (% RSD). Precision of the assay was measured by the percent coefficient of variation over different concentration levels. The acceptance criteria for within and between batch precision were 20% or better for LOQQC and 15% or better for other non-zero concentrations. The accuracy of the assay was defined as the ratio of calculated mean values of the quality control sample to their respective nominal values, expressed as percentage and the criteria for accuracy was 100 ± 20% or better for LOQQC and 100 ± 15% or better for other concentrations. For Buproprion intra-day experiments, the precision ranged from 0.88% to 5.12% and the accuracy ranged from 95.93% to 103.17% whereas for the inter-day experiments, precision ranged from 1.24 % to 6.41 % and the accuracy ranged from 98.16 % to 104.61 %. For Hydroxybuproprion intra-day experiments, the precision ranged from 1.47% to 4.43% and the accuracy ranged from 92.67% to 107.34% whereas for the inter-day experiments, precision ranged from 2.06 % to 7.64 % and the accuracy ranged from 92.35 % to 108.00 %.

Recovery

Recovery of Buproprion and Hydroxybuproprion from the extraction procedure was determined by preparing aqueous recovery comparison samples at

LQC, MQC and HQC levels (representing 100 % extraction).The aqueous recovery samples (LQC, MQC and HQC) of Bupropion and Hydroxybupropion was estimated by analysis and comparison against 6 sets of LQC, MQC and HQC. Similarly, recovery of internal standard was compared at LQC, MQC and HQC level. The mean % recovery of Bupropion was found to be 58.443 % with a precision of 1.01 % whereas the mean % recoveries of Hydroxybupropion and Bupropion D9 were found to be 62.327 % and 66.513 % with a precision of 2.99 % and 3.91 % respectively

Stability study

Stability studies were performed to evaluate the Buprorion and Hydroxybuprorion stability in stock solution, in matrix under different conditions (bench top stability, auto sampler stability, freeze thaw stability, long term stability - 65°C ± 10°C & long term stability - 22°C ± 5°C, dry extract stability, wet extract stability in refrigerator and at bench top, blood stability). Stock solutions of Buprorion, Hydroxybuprorion and ISTD were stable at room temperature for 22 hrs, 57 min and in refrigerator at 2-8°C for 6 days. Stock dilution of Buprorion, Hydroxybuprorion and ISTD were stable at room temperature for 22 hrs, 15 min Spiked Buprorion and Hydroxybuprorion were stable in human plasma (bench top stability) at room temperature for 15 hrs 23 min. Buprorion and Hydroxybuprorion were found stable in the final extract for analysis while placed in the autosampler up to 48 hrs, 57 min. Buprorion and Hydroxybuprorion were found to be stable for at least five freeze and thaw cycles. The Buprorion and Hydroxybuprorion spiked plasma samples stored below -65°C and -22°C for long- term stability experiment were found to be stable for 297 days. The processed long term stability -65°C ± 10°C and -22°C ± 5°C quality control samples and freshly spiked quality control samples were quantified against the freshly spiked calibration curve standards. Dry Extract Stability of Bupropion and Hydroxybupropion were demonstrated for 50 hours, 50 minutes. Wet Extract Stability of Bupropion and Hydroxybupropion were demonstrated for 51 hours, 28 minutes whereas the wet extract bench top stability of Bupropion and Hydroxybupropion were demonstrated for 5 hours, 3 minutes. Blood stability for spiked samples is carried out to assess the stability of the analyte(s) in blood. Blood stability was performed by the preparation of six sets of quality control samples Medium Quality Control (MQC) and High Quality Control samples (HQC) by spiking 2 % of MQC and HQC aqueous dilution in blood which were kept on the bench at room temperature for approximately 1 hour. Samples were then centrifuged at 4000 rpm at 4°C for 5 minutes to separate the plasma, then plasma samples were processed and analyzed along with freshly spiked CC, and QC samples (MQC and HQC) prepared and separated in a similar way. Blood stability is expressed as the percentage change between the fresh and the stability samples. The % change for Bupropion were -1.62 % (MQC) and 0.18 % (HQC) whereas the % change for Hydroxybupropion were -0.15 % (HQC) and -0.02 % (MQC).Additionally reagent stability was carried out to confirm the stability of the reagents during the period of their use in analysis. For estimating reagent Stability, six sets of quality control samples (LQC and HQC) were processed in aged solutions that were kept on bench at room temperature for the stability duration and they were quantified against a calibration curve standards, which was processed using fresh solutions. The precision and accuracy of the quality control samples is used as an estimate of the reagent stability. Reagents used in analysis were found stable for duration of 7 days.

Matrix effect assessment

Matrix effect was evaluated to demonstrate that no considerable endogenous contribution from human

blank plasma affects the measurement of the analytes. The matrix effect on the ionization of Buproprion and Hydroxybuproprion in the LC MS-MS method was determined by processing of six altered set of plasma lots in duplicate. The neat (non matrix based) samples were prepared using mobile phase, aqueous dilutions of Bupropion and Hydroxy Bupropion at the LQC and HQC levels and IS dilution to give concentration within the level of extracted value. The processed matrix effect blanks samples were reconstituted with neat samples. The matrix effect on the estimation of Bupropion and Hydroxybuproprion were determined as the % RSD (relative standard deviation) of the matrix factor or the variability of the matrix factor. Matrix factor is given by the comparison of the area response of the blank samples with the neat samples. The variability of matrix factor (reported as % CV of matrix factor) was 3.44 % (HQC) and 5.26 % (LQC) for Bupropion. The variability of matrix factor (reported as % CV of matrix factor) was 1.75 % (HQC) and 4.60 % (LQC) for Hydroxy Bupropion, whereas it was 1.76 % (LQC) and 4.32 % (HQC) for Bupropion D9

Effect of potentially interfering drug

Effect of potentially interfering drug was evaluated to demonstrate the effect of potential drugs that could affect the analysis (by creating any interference at the retention levels of analyte and internal standard) because of their administration to the study volunteers during the clinical phase of the study. Potentially co-administered drugs prepared at therapeutic concentrations were tested to verify their interfering potential at the RT of an analyte and the IS. PID LLOQ samples were prepared by the spiking of 2 % of the respective PIDs at their expected Cmax concentration and 2 % of Lower Limit of Quantification (LLOQ) in blank matrix. These samples were analysed against a calibration curve. The effect of potentially interfering drugs (PID) (Ibuprofen, Caffeine, Acetaminophen and Acetyl

salicylic acid) on Bupropion and Hydroxy Bupropion analysis was performed. The back calculated concentrations of QC sample spiked with PID were found to be within ± 15 % of the actual concentration of the QC sample for Bupropion and Hydroxy Bupropion. Hence, the above-mentioned PIDs have no effect on the analysis of Bupropion and Hydroxy Bupropion.

Application of the method

The validated method was successfully applied to quantify concentration of Buproprion and Hydrxybuproprion in human plasma samples during bioequivalence study, Bupropion Hydrochloride Extended Release Tablets (XL) 150mg of test product with reference Product (Bupropion Hydrochloride Extended Release Tablets) 150mg in Healthy Adult, human Subjects, under fasting and fed conditions. Representative mean plasma concentration profile curve of test and reference given in **Fig. 3.**

CONCLUSION

A highly selective and rapid LC-ESI MS-MS method for the determination of Buproprion and Hydrxybuproprion in human plasma has been developed and validated with a lower limit of quantification of ($1.00ng mL^{-1}$) for Buproprion and Hydroxybuproprion ($3.00ng mL^{-1}$). The underlying advantage of this validated method was that it uses only 200μL of human plasma thus minimizing untoward matrix effect, also the injection volume was restricted to 5μL, *i.e.* only 0.025ng (Buproprion) and 0.075ng (Hydroxybuproprion) was injected onto the column at LLOQ level, which eliminate any chances of response saturation at the ULOQ ranges. Further, the added advantage of solid phase extraction method provides efficient sample cleanup, higher sensitivity with negligible matrix effect with shorter (3.0 min) analytical run time to ensure high throughput during analysis.

REFERENCES

1) Wilkes S., The use of bupropion SR in cigarette smoking cessation, Int J Chron Obstruct Pulmon Dis 2008; 3(1): 45-53.

2) Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Mürdter TE, Roots I, Brockmöller J., Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6, Pharmacogenetics 2003; 13(10): 619-26.

3) Parekh JM, Sutariya DK, Vaghela RN, Sanyal M, Yadav M, Shrivastav PS., Sensitive, selective and rapid determination of bupropion and its major active metabolite, hydroxybupropion, in human plasma by LC-MS/MS: application to a bioequivalence study in healthy Indian subjects, Biomed Chromatogr. 2012; 26(3): 314-26.

4) Borges V, Yang E, Dunn J, Henion J., High-throughput liquid chromatography-tandem mass spectrometry determination of bupropion and its metabolite in human, mouse and rat plasma using a monolithic column, J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 804(2): 277-87.

5) Hu L, Wang Z, Xu R, Ma J, Wang X, Zhang X., Determination of bupropion and its metabolite in rat plasma by LC-MS and its application to pharmacokinetics, Pharmazie 2011; 6(12): 924-928.

6) Yeniceli D, Sener E, Korkmaz OT, Doğrukol-Ak D, Tuncel N., A simple and sensitive LC-ESI-MS (ion trap) method for the determination of bupropion and its major metabolite, hydroxybupropion in rat plasma and brain microdialysates, Talanta. 2011; 84(1): 19-26.

7) Loboz KK, Gross AS, Ray J, McLachlan AJ., HPLC assay for bupropion and its major metabolites in human plasma., J Chromatogr B Analyt Technol Biomed Life Sci. 2005; 823(2): 115-21.

8) White Paper on Recent Issues in Bioanalysis and Regulatory Findings from Audits and Inspections. Bioanalysis 2011; 3(18), 2081-2096.

9) Guidance for Industry: Bioanalytical Method Validation US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research (CDER), Rockville, MD