Identification of non-reported bupropion metabolites in human plasma

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ABSTRACT: Bupropion and its three active metabolites exhibit clinical efficacy in the treatment of major depression, seasonal depression and smoking cessation. The pharmacokinetics of bupropion in humans is highly variable. It is not known if there are any non-reported metabolites formed in humans in addition to the three known active metabolites. This paper reports newly identified and non-reported metabolites of bupropion in human plasma samples. Human subjects were dosed with a single oral dose of 75 mg of an immediate release bupropion HCl tablet. Plasma samples were collected and analysed by LC–MS/MS at 0, 6 and 24 h. Two non-reported metabolites (M1 and M3) were identified with mass-to-charge (m/z) ratios of 276 (M1, hydration of bupropion) and 258 (M3, hydroxylation of threo/erythrohydrobupropion) from human plasma in addition to the known hydroxybupropion, threo/erythrohydrobupropion and the glucuronidation products of the major metabolites (M2 and M4–M7). These new metabolites may provide new insight and broaden the understanding of bupropion's variability in clinical pharmacokinetics. © 2016 The Authors *Biopharmaceutics & Drug Disposition* Published by John Wiley & Sons Ltd.

Key words: bupropion; metabolite identification; LC–MS/MS

Introduction

Bupropion is prescribed for the treatment of major depressive disorder, seasonal affective disorder and smoking cessation [1–3]. It is reported that bupropion is a non-selective inhibitor of the dopamine and norepinephrine transporter as well as an antagonist of the neuronal nicotinic acetylcholine receptor [4,5].

Bupropion is extensively metabolized to three known active metabolites, hydroxybupropion and the diasteroisomers threohydrobupropion and erythrohydrobupropion. Using an animal model it was shown that these metabolites may exhibit as much as 25–50% potency compared with the parent drug [6,7]. The metabolite hydroxybupropion is formed by cytochrome P450 2B6 (CYP2B6) while threo/erythrohydrobupropion are formed by carbonyl reductase (CR) [8–10]. It is reported that there are multiple enzymes involved in the CR pathway

including 11-β HSD and AKR7 family [8,11,12]. Both bupropion and its known major active metabolites display long half-lives; bupropion ~21 h, hydroxybupropion ~20 h, and threo/erythrohydrobupropion ~33–36 h [13]. Additionally, the C_{max} of threohydrobupropion and hydroxybupropion is ~3 and ~4–5 times higher in plasma than that of bupropion, respectively [13]. Recent reports show that the major metabolites (hydroxybupropion and threo/erythrohydrobupropion) undergo glucuronidation [14].

About 87% of bupropion and its metabolites are excreted in the urine where only 0.5% is excreted unchanged, and 10% of bupropion is eliminated in the feces where only 0.1% is unchanged in humans after an oral dose [15,16]. However, one study investigated the elimination and recovery of bupropion after a single oral dose in urine from humans, where the data indicated that bupropion comprised 0.6% and hydroxybupropion comprised 2.8% of the total recovery; in total these two analytes constituted less than 3.4% of the total drug recovered [17]. Although threohydrobupropion or erythrohydrobupropion were not quantified in this study, their abundance is usually lower than that of hydroxybupropion [13,18]. The low recovery of bupropion and hydroxybupropion suggests that other metabolites might be formed. In addition, the pharmacokinetics and efficacy of bupropion is highly variable in clinical studies [18,19]. It is suspected that other non-reported metabolites may be formed. Since the three known major metabolites are considered active, understanding the metabolic pathway and discovery of new metabolites may shed light on the variability of pharmacokinetics and the efficacy of bupropion.

Healthy human subjects were administered a single dose of immediate release bupropion HCl (75 mg). Plasma samples were collected at 0, 6 and 24 h for analysis. To identify additional metabolites, enhanced mass spectrometry (EMS), multiple ion monitoring (MIM) and precursor ion scans were used to analyse the plasma samples. In all four subjects, M1–M7, were identified in addition to the three major known active metabolites. M1 is formed from the hydration of the benzene ring of bupropion and M3 results from further oxidation of threo/erythrohydrobupropion or reduction of hydroxybupropion. Both M1 and M3 have unknown activity although it is possible that they could be active. These data provide new insight on bupropion's metabolism *in vivo*, which may contribute to its variability in pharmacokinetics and efficacy. Further studies are warranted to assess the pharmacological activity of these metabolites.

Methods and Materials

Reagents

Bupropion HCl and venlafaxine HCl (internal standard; IS) were purchased from Sigma (St Louis, MO). Hydroxybupropion was purchased from Cayman Chemicals (Ann Arbor, MI). Threohydrobupropion and erythrohydrobupropion were purchased from Torando Chemical Company (Torando, Canada). Methanol, LC–MS/MS grade, was purchased from Fischer Sciences (Hampton, NH).

Plasma sample extraction

All plasma samples were obtained in accordance to the University of Michigan Institutional Review Board (HUM00081894) and the Food & Drug Administration Institutional Review Board/ Research Involving Human Subjects Committee (RIHSC #13-087D). Healthy participants voluntarily took a single dose of an immediate release 75 mg of bupropion HCl tablet. Blood samples were collected in EDTA K2 pretreated tubes from four participants at 0, 6 and 24 h, then immediately centrifuged at 4 °C for 10 min at 14000 rpm. The plasma (supernatant) was then transferred to another cryogenic tube and stored at −80 °C until analysis. Plasma was diluted 1:3 in methanol and vortexed for 1 min. The samples were centrifuged at 14000 rpm for 10 min at 4 °C. The supernatant was transferred to another tube and evaporated using nitrogen gas and reconstituted in 120 μl of 20% methanol in water. The samples were then analysed by LC–MS/MS to monitor metabolites.

Liquid chromatography-mass spectrometry method

The LC–MS/MS analysis was conducted using a Shimadzu SIL-20ACHT HPLC system coupled with an API 4500 mass spectrometer (Applied Biosystems, MDS Sciex Toronto, Canada), equipped with an API electrospray ionization (ESI) source. The LC–MS/MS (or LC–MS) was operated at positive ESI ionization mode. The Supelco C18 (150 × 4.6 mm i.d., 5 μm) column was purchased from Sigma-Aldrich and used for separation. The LC separation condition was optimized with a blank plasma sample spiked with bupropion and its three known metabolites. The mobile phases consisted of 0.04% formic acid in purified water (A) and 0.04% formic acid in methanol (B). The gradient was run at 0–2 min 2% (B), an increase to 32% (B) from 2–32 min, followed by 90% (B) at 32–36 min, and finally decreased back to 2% (B) at 36–39.5 min with a flow rate of 1 ml/min. The EMS, the precursor ion scan and the MIM scan were used as the survey scan to identify the metabolites of bupropion. Either information dependent acquisition (IDA) was used to collect fragmentation of metabolite candidates or single experiment of the enhanced product ion scan (EPI) was applied to acquire fragmentations of metabolites after running the survey scan.

The ion spray voltage of QTrap (ABI) was set at 5500 V with a spray temperature of 600 °C, curtain gas of 30 psi, gas 1 and gas 2 of 60 psi. The declustering potential (DP) was set at 51 V. The collision energy was kept at 5 eV for the survey scan of EMS & MIM and 25 eV for the precursor ion scan and EPI fragmentation.

Additionally, a more thorough analysis was performed as described below to examine the isotopic patterns of the newly identified metabolites. High resolution LC–MS analyses were performed using an HPLC system (1200 series, Agilent Technologies) coupled to a 6520 Accurate-Mass Q-TOF (Agilent Technologies) operated in a positive electrospray ionization mode. The ESI conditions were gas temperature 325 °C, drying gas 5 l/min, nebulizer 30 psig, fragmentor 150 V and skimmer 65 V. The instrument was set to acquire over the m/z range 50 to 3200 with an acquisition rate of 2 spectra/s.

Data analysis

The analytical data were processed by the software Analyst version 1.6.2 (Applied Biosystems, Foster City, CA). The data were also analysed in Lightsight version 2.3.0 (Applied Biosystems). ChemBioDraw Ultra version 13.0 (CambridgeSoft) was used to draw structures.

Results

Bupropion and its metabolites

Figure 1 highlights the structures of the currently known metabolites of bupropion (hydroxybupropion and threo/erythrohydrobupropion) and seven metabolites identified in this study from human plasma. Bupropion is metabolized by both CYP2B6 and CR to form hydroxybupropion and threo/erythrohydrobupropion, respectively [8–10]. It is also known that threo/erythrohydrobupropion can further undergo glucuronidation [20]. In addition to these metabolites, M1 and M3 were detected in human plasma in this study and are shown as non-reported metabolites. Figure 2 illustrates the daughter ions of bupropion (2A) and the daughter ions of three known metabolites; hydroxybupropion (2B), threohydrobupropion (2C) and erythrohydrobupropion (2C) that were acquired by direct MS infusion using authentic standards. Threohydrobupropion and erythrohydrobupropion have the same product ion spectra and fragments. The fragmentation of bupropion and its three metabolites were proposed based on their product ion spectra. The characteristic daughter ions included m/z ratios of 103, 131, 139, 151, 166, 167, 168, 184 and 238, which served as precursor ions for the survey scan of the precursor ion scan.

Identification of non-reported bupropion's metabolites

Figure 3 indicates the extracted ion chromatograms of all metabolites detected in human plasma after a single oral dose of bupropion. Figure 3A shows the relative intensity and retention times for all metabolites detected, including bupropion and the previous three known metabolites in one MIM method after all survey scans
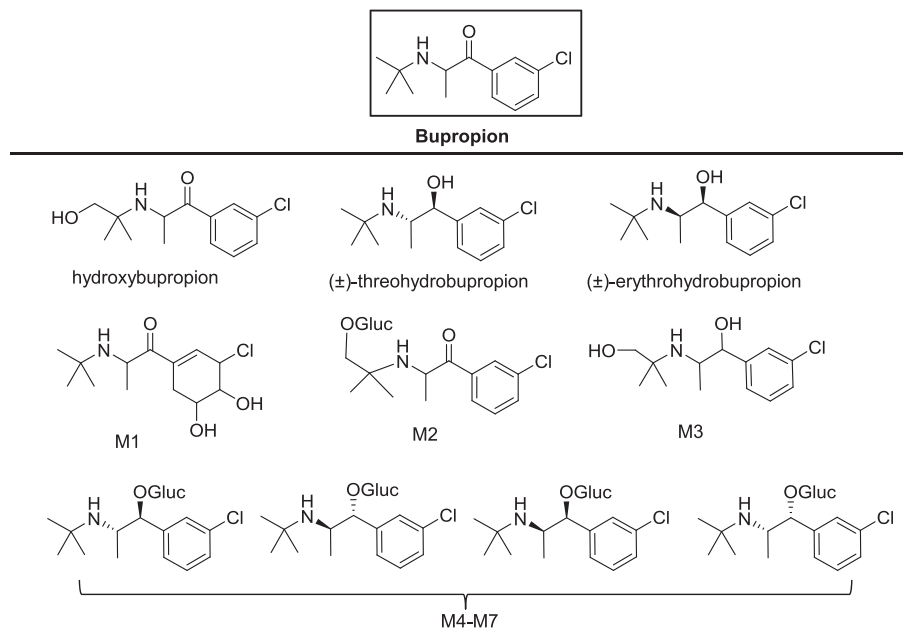
Figure 1. Bupropion and its metabolites found in human plasma. Bupropion has been reported to be metabolized by both CYP2B6 and carbonyl reductase to form hydroxybupropion and threo/erythrohydrobupropion, respectively. Seven other metabolites were identified in human plasma after a single oral dose of bupropion; hydration of bupropion (M1), glucuronidation of hydroxybupropion (M2), hydroxylation of threo/erythrohydrobupropion (M3) and glucuronidation of threo/erythrohydrobupropion (M4–M7)

were inspected. Figure 3B–E shows the extracted ion chromatograms of seven metabolites detected in plasma, corresponding to m/z ratios of (B) M1: 276, (C) M2: 432, (D) M3: 258, (E) M4–M7: 418. The M1 metabolite elution was quite early. M2 and M3 were eluted right after hydroxybupropion. Multiple peaks with the same $m/z = 418$ were observed (Figure 3E) and represented by M4, M5, M6 and M7. These four peaks all had the same product ion spectra, suggesting that stereochemistry might be involved. Together, the extracted ion chromatogram results suggested seven metabolites in addition to hydroxybupropion and threo/erythrohydrobupropion.

Structure determination of new bupropion metabolites

To determine the structures of the newly identified metabolites, the product ion spectra were evaluated (Figure 4). The m/z ratio of M1 ($m/z = 276$) increased 36 Daltons (Da) compared with that of bupropion ($m/z = 240$). As shown in Figure 4A, the fragments of the characteristic ions that were consistent with bupropion were m/z ratios of 167, 184 and 240. The M1 metabolite was further investigated with a high resolution mass spectrometer. The accurate mass (m/z) of M1 that was measured was 276.1356 as shown in Figure 5. This molecular ion also showed the characteristic isotopic pattern of chlorine atoms. The monoisotopic ion of 276 has an intensity three times that of isotopic ion 278 as shown in Figure 5, confirming the metabolite was indeed real and not an artifact. The predicted formula for this metabolite was $C_{13}H_{22}ClNO_3$ with a mass accuracy of 2 ppm, which contains four more hydrogen atoms and two more oxygen atoms than bupropion. These additional atoms were assigned to the benzyl ring of bupropion as shown in the proposed structure in Figure 4A.

M2 showed an m/z ratio of 432, which had 176 Da more than hydroxybupropion (Figure 4 B). In addition, its fragments contained the same characteristic ions of 166, 184 and 256 as seen in hydroxybupropion, suggesting glucuronidation of hydroxybupropion.

M3 ($m/z = 258$) increased in 16 Da compared with threo/erythrohydrobupropion and 18 Da more than bupropion. The fragments of M3 (Figure 4C)
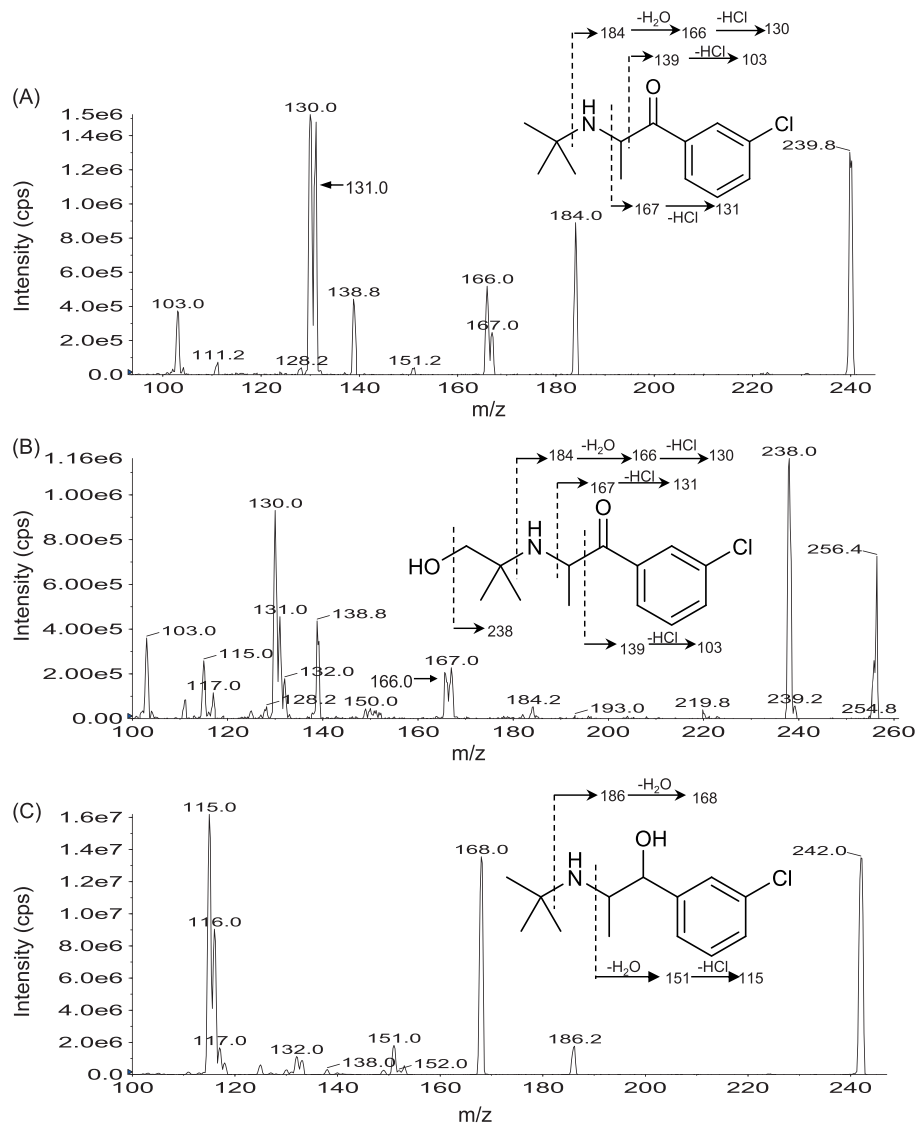
Figure 2. Proposed fragmentation of bupropion and its metabolites. Authentic standards were directly infused to acquire product ion spectra of (A) Bupropion (m/z 240); (B) Hydroxybupropion (m/z = 256); (C) Threo/Erythrohydrobuproprion (m/z = 242). The structure indicates how these fragmentations occur on each molecule

had the same characteristic ions as seen in threo/erythrohydrobupropion; 151, 168 and 186. The fragment of 186 corresponded to a loss of the tert-butyl group and therefore no modification could occur in the fragmented structure of 186. This suggested that 16 Da affected the tert-butyl group of threo/erythrohydrobupropion. The product ion of m/z 240 of M3 confirmed this position. The 16 Da increase was proposed as hydroxylation to threo/erythrohydrobupropion. In Figure 3D, in

addition to the peak for M3, a large peak was observed with the same parent ion of 258. This peak eluted out earlier than M3 and at same retention time as hydroxybupropion (22 min). The product ion spectrum of this peak confirmed that it is the hydroxybupropion isotopic ion containing [37]Cl.

Finally, the last metabolite m/z = 418, had 176 Da more than threo/erythrohydrobupropion, and it also had characteristic ions of m/z 151, 168 and 186 as seen in threo/erythrohydrobupropion,
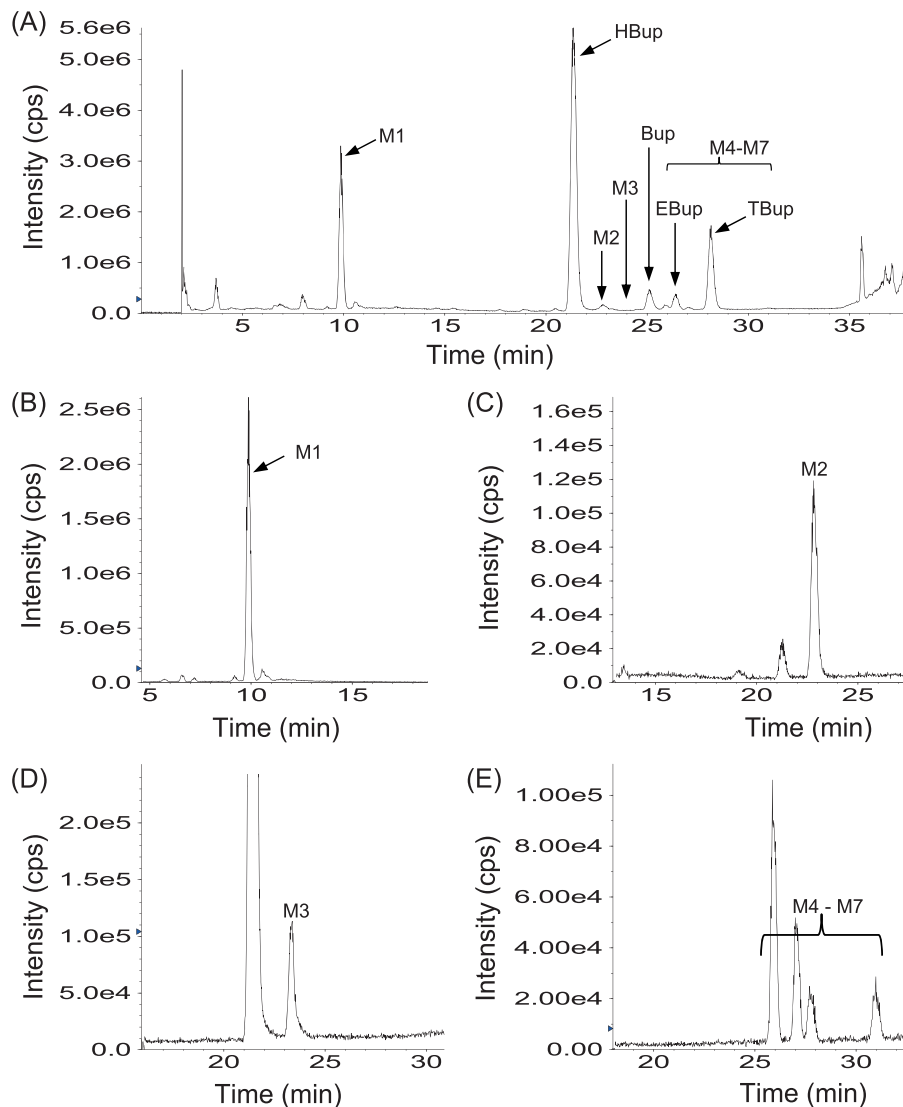
Figure 3. Extracted ion chromatograms for bupropion metabolites found from human plasma. (A). All metabolites observed including; hydroxybupropion (Hbup), threo/erythrohydrobupropion (TBUP/EBUP), and M1–M7; (B) M1 ($m/z = 276$); (C) M2 ($m/z = 432$); (D) M3 ($m/z = 258$); (E) M4–M7 ($m/z = 418$). For the metabolites M4–M7, the same m/z value of 418 was observed

suggesting glucuronidation (Figure 4D). This observation was consistent with Figure 3E producing four peaks. It can be postulated that two of these peaks were from the conjugation of chiral β-D glucuronic acid to a hydroxyl group of two threohydrobupropion enantiomers, respectively, and the other two were from the β-D glucuronic acid conjugation of two erythrohydrobupropion enantiomers, respectively. The conjugation of chiral glucuronic acid introduced another chiral center to the enantiomers and resulted in four diasteroisomers. These conjugates could be separated on the column showing four distinct peaks.

The isotopic distribution of three other metabolites through EMS full scans with a narrow mass range was also examined. As shown in Supplementary Figure S1, mass spectra of M2, M3 and M4–M7 all have a relative isotopic abundance of ^{35}Cl and ^{37}Cl, which further confirmed these three metabolites came from bupropion.
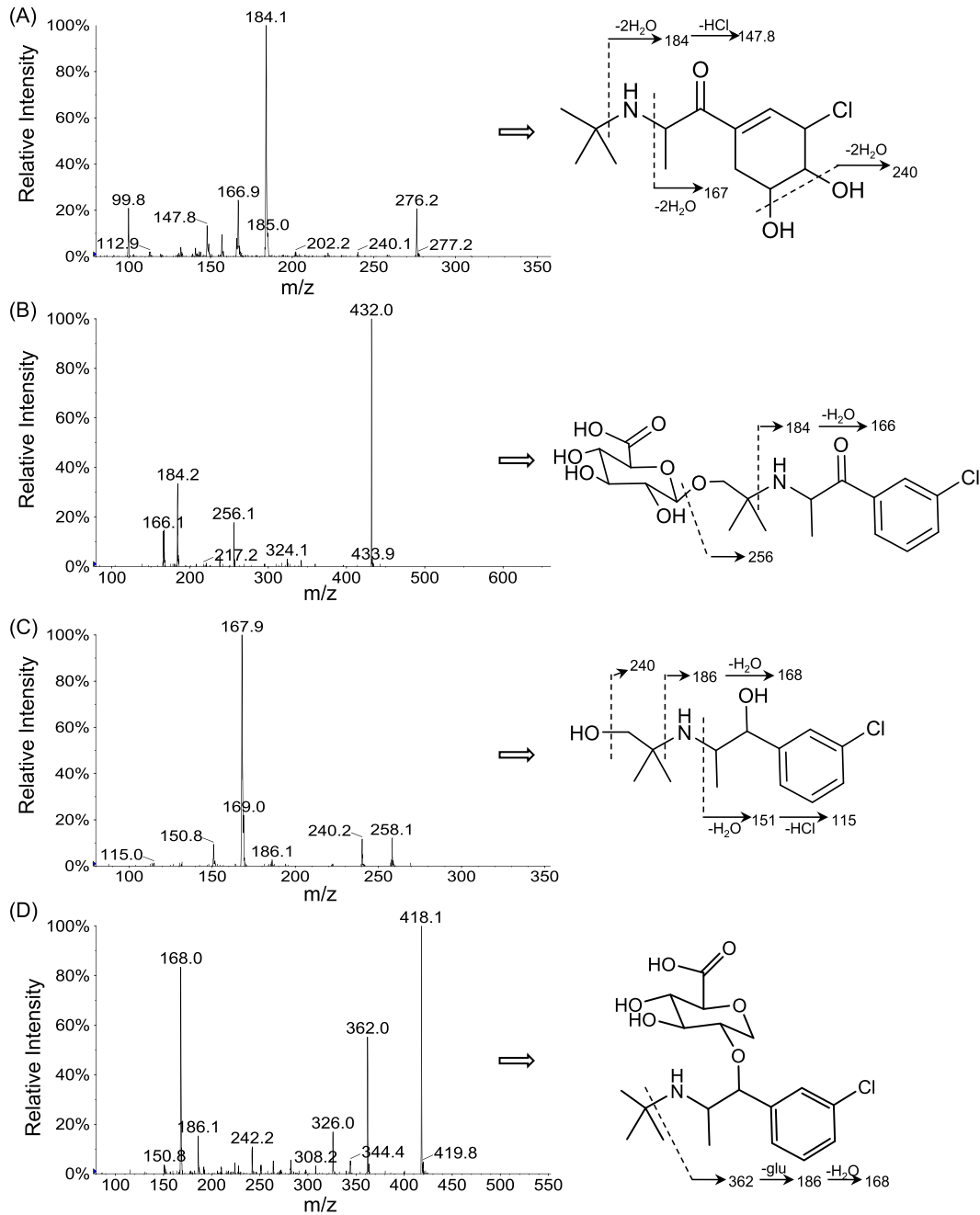
Figure 4. Proposed fragmentation of bupropion metabolites found from human plasma. The spectra indicated the fragmentation of each of the bupropion metabolites detected. Their structures were proposed: (A) M1, hydration of bupropion (m/z = 276); (B) M2, glucuronidation of hydroxybupropion (m/z = 432); (C) M3, oxidation of threo/erythrohydrobupropion (m/z = 258); (D) M4, glucuronidation of threo/erythrohydrobupropion (m/z = 418). M4–M7 have same product ion spectra, therefore only one spectrum is shown here
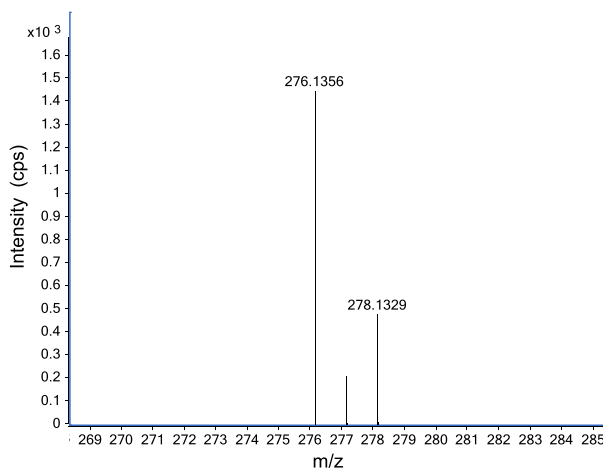
Figure 5. High resolution mass and isotopic pattern of newly found metabolite, m/z = 276. The predicated formula of this metabolite is $C_{13}H_{22}Cl\,NO_3$ with a mass accuracy of 2 ppm. The isotopic pattern of ^{35}Cl and ^{37}Cl offers strong evidence that this metabolite is a product of bupropion

Relative quantification of new metabolites in human plasma

To assess each metabolite formation in each subject at the two time points of 6 and 24 h, the peak areas (counts) were compared (Figure 6). The peak areas for each metabolite for the four subject samples are illustrated in Figure 6 by the m/z ratio. The time points at 6 vs. 24 h were compared in order to assess the relative amount of metabolite formed. Over time, the majority of the metabolites had a higher peak area at 6 h compared with at 24 h (Figure 6A–G). There were variations in the peak area for each metabolite formation between subjects, with M1 showing the largest variability. However, these data suggest that all seven metabolites form in vivo.

Discussion

The biotransformation of bupropion involves multiple enzymes that are expressed at different levels in both the gastrointestinal tract and the liver [21,22]. Since bupropion HCl is clinically available in multiple formulations (immediate release, sustained release or extended release) at various doses (strengths 75–300 mg), it is likely that these factors may influence the amount of metabolites formed in the liver and gastrointestinal (GI) tract. Therefore, we intended to

investigate the complete biotransformation of bupropion in vivo.

This study analysed human plasma after a single oral dose of a 75 mg bupropion HCl tablet. Seven metabolites were identified including two non-reported metabolites for bupropion; hydration of bupropion (M1) and oxidation of threo/erythrohydrobupropion (M3). Hydration of bupropion was assigned to the benzyl ring of bupropion since only the ring has unsaturated bonds for the addition of hydrations (Figure 4A). The fact that the retention time was much shorter compared with the other metabolites of bupropion provided evidence of the hydration of bupropion, since more polar compounds typically result in shorter retention times. Much to our surprise, only one peak for hydroxylation of threo/erythrohydrobupropion was observed (M3). One explanation is that the second peak was too low to observe, which seems sensible considering how low the abundance of erythrohydrobupropion is in human plasma. The M4–M7 metabolites share the same m/z ratio and fragmentation pattern, which indicates selective stereochemistry that is expected from the diastereoisomers of threo/erythrohydrobupropion through conjugation to β-D glucuronic acid. The isotopic pattern of chlorine was used to distinguish the metabolite candidates that may have been detected but were not a result of bupropion metabolism [23]. Bupropion contains one chlorine
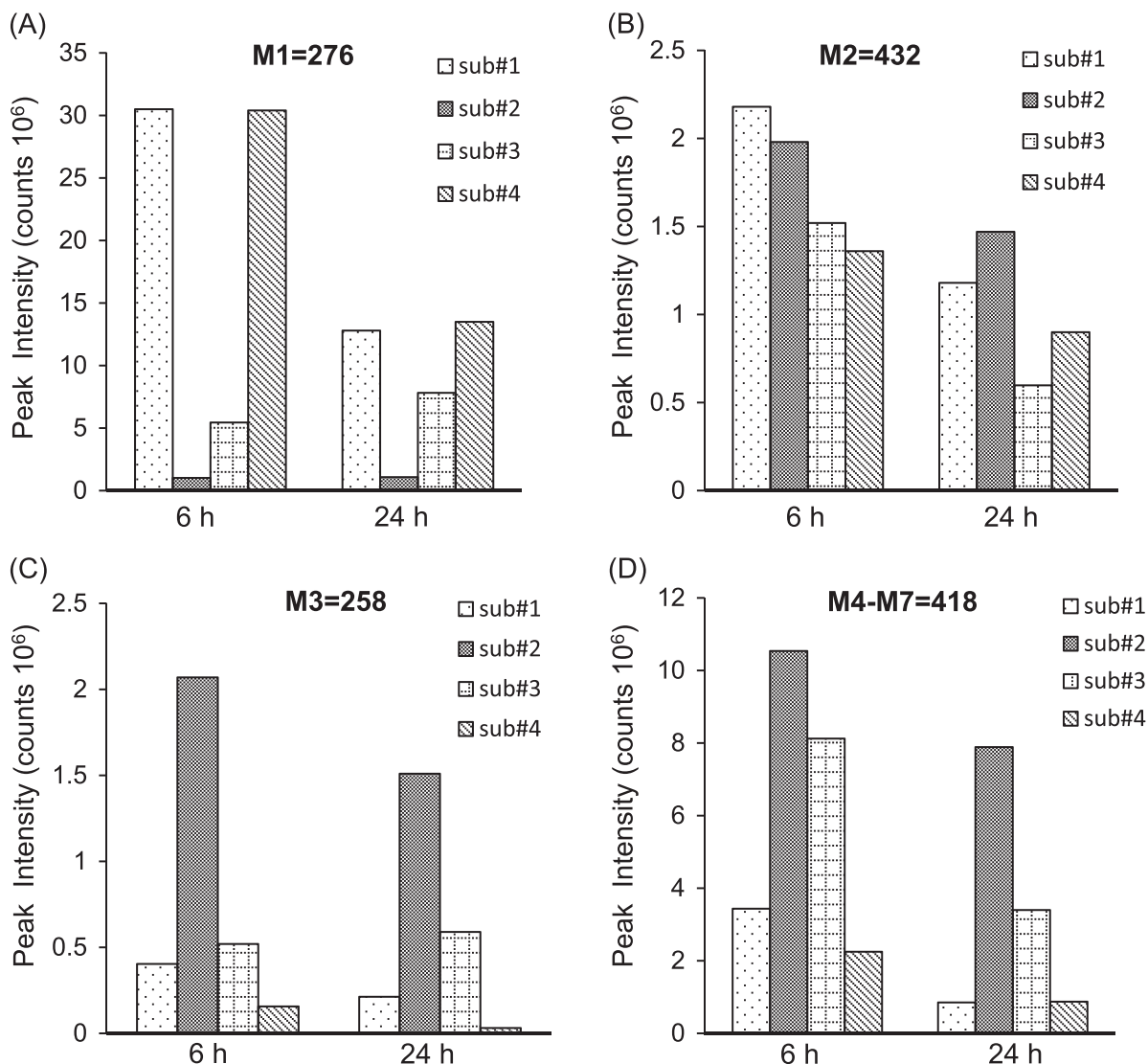
(A)

(B)

(C)

(D)

Figure 6. Relative intensity of metabolites newly found in four human subjects. For comparison purposes, the peak area of each new metabolite that formed in the plasma sample for each subject is shown at 6 h and 24 h. (A) M1 with m/z of 276; (B) M2 with m/z of 432; (C) M3 with m/z of 258 and (D) the sum of M4–M7 peak areas with m/z of 418

atom on the benzyl ring, which assists in the identification of metabolites from complex plasma samples. The isotopic pattern of the chlorine atom in Figure 5 clearly demonstrated that the new metabolites are produced via biotransformation of bupropion.

Gufford *et al.* has recently proposed several UGT enzymes that might be responsible for the formation of a glucuronidation metabolite for bupropion, these same glucuronidation products were consistent with what was observed in this study [14]. However, the Gufford *et al.* study analysed urine and not plasma. Therefore, M1 or M3 may not have been excreted in urine or these metabolites may not have been identified using only multiple reaction monitoring (MRM). In addition to the MRM scanning method Gufford *et al.* used, samples in this study also were tested using a precursor ion scan, MIM and EMS, allowing for the detection of M1 and M3.

While all seven metabolites formed in four different individuals, the peak area of these metabolites varied quite a bit from individual to individual. The known primary enzymes responsible for the conversion of bupropion to form hydroxybupropion and threo/erythrohydrobupropion are highly polymorphic. CYP2B6 can have a 20–250 fold variability in terms of expression and activity and in addition is expressed at different levels in multiple tissues, including the GI tract and the liver [19,21,24–27]. Carbonyl reductase enzymes have also been found to be highly polymorphic with varying activity [28,29]. Similarly, studies have shown the uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes, which are responsible for glucuronidation reactions, have high variability in terms of activity, which might lead to a functional impact [30,31]. Therefore, it is reasonable to suspect that polymorphs can explain the intersubject variability observed amongst these four subjects.

Previous *in vitro* results have suggested that other possible phase I metabolism enzymes might be involved in the biotransformation of bupropion and major metabolites, specifically hydroxybupropion including: CYP2E1 CYP2C19 and CYP3A4 *in vitro* [25,32,33], however, this has not been explored *in vivo*. These phase I metabolism enzymes may also be candidates for forming these new metabolites. The metabolites identified in this study may exhibit activity, however, further investigations would be necessary to determine if these metabolites are indeed active. Nevertheless, the identification of M1–M7 may shed light for the further variability of bupropion in terms of pharmacokinetics and efficacy.

Conclusion

Seven metabolites of bupropion in human plasma samples were detected after a single dose of bupropion HCl (IR 75 mg). Two non-reported metabolites (M1 and M3) were identified with mass-to-charge (m/z) ratios of 276 (M1, hydration of bupropion) and 258 (M3, hydroxylation of threo/erythrohydrobupropion) in addition to the known hydroxybupropion, threo/erythrohydrobupropion and the glucuronidation products of the major metabolites (M2 and M4–M7).

Conflict of Interest

There is no conflict of interest with the authors.

References

1. Desmarais JE, Beauclair L, Margolese HC. Switching from brand-name to generic psychotropic medications: a literature review. *CNS Neurosci Ther* 2011; **17**(6): 750–760.
2. Fava M, Rush AJ, Thase ME, *et al.* 15 years of clinical experience with bupropion HCl: from bupropion to bupropion SR to bupropion XL. *Prim Care Companion J Clin Psychiatry* 2005; **7**(3): 106–113.
3. Reese MJ, Wurm RM, Muir KT, Generaux GT, St John-Williams L, McConn DJ. An *in vitro* mechanistic study to elucidate the desipramine/bupropion clinical drug–drug interaction. *Drug Metab Dispos* 2008; **36**(7): 1198–1201.
4. Dwoskin LP, Rauhut AS, King-Pospisil KA, Bardo MT. Review of the pharmacology and clinical profile of bupropion, an antidepressant and tobacco use cessation agent. *CNS Drug Rev* 2006; **12**(3–4): 178–207.
5. Stahl SM, Pradko JF, Haight BR, Modell JG, Rockett CB, Learned-Coughlin S. A review of the neuropharmacology of bupropion, a dual norepinephrine and dopamine reuptake inhibitor. *Prim Care Companion J Clin Psychiatry* 2004; **6**(4): 159–166.
6. Bondarev ML, Bondareva TS, Young R, Glennon RA. Behavioral and biochemical investigations of bupropion metabolites. *Eur J Pharmacol* 2003; **474**(1): 85–93.
7. Damaj MI, Carroll FI, Eaton JB, *et al.* Enantioselective effects of hydroxy metabolites of bupropion on behavior and on function of monoamine transporters and nicotinic receptors. *Mol Pharmacol* 2004; **66**(3): 675–682.
8. Connarn JN, Zhang X, Babiskin A, Sun D. Metabolism of bupropion by carbonyl reductases in liver and intestine. *Drug Metab Dispos* 2015; **43**(7): 1019–1027.
9. Hesse LM, Venkatakrishnan K, Court MH, *et al.* CYP2B6 mediates the *in vitro* hydroxylation of bupropion: potential drug interactions with other antidepressants. *Drug Metab Dispos* 2000; **28**(10): 1176–1183.

DOI: 10.1002/bdd

10. Skarydova L, Tomanova R, Havlikova L, Stambergova H, Solich P, Wsol V. Deeper insight into the reducing biotransformation of bupropion in the human liver. *Drug Metab Pharmacokinet* 2014; **29**(2): 177–184.

11. Meyer A, Vuorinen A, Zielinska AE, *et al.* Formation of threohydrobupropion from bupropion is dependent on 11beta-hydroxysteroid dehydrogenase 1. *Drug Metab Dispos* 2013; **41**(9): 1671–1678.

12. Molnari JC, Myers AL. Carbonyl reduction of bupropion in human liver. *Xenobiotica* 2012; **42**(6): 550–561.

13. Jefferson JW, Pradko JF, Muir KT. Bupropion for major depressive disorder: pharmacokinetic and formulation considerations. *Clin Ther* 2005; **27**(11): 1685–1695.

14. Gufford BT, Lu JB, Metzger IF, Jones DR, Desta Z. Stereoselective glucuronidation of bupropion metabolites *in vitro* and *in vivo*. *Drug Metab Dispos* 2016; **44**(4): 544–553.

15. Findlay JW, Van Wyck Fleet J, Smith PG, *et al.* Pharmacokinetics of bupropion, a novel antidepressant agent, following oral administration to healthy subjects. *Eur J Clin Pharmacol* 1981; **21**(2): 127–135.

16. Schroeder DH. Metabolism and kinetics of bupropion. *J Clin Psychiatry* 1983; **44**(5 Pt 2): 79–81.

17. Coles R, Kharasch ED. Stereoselective analysis of bupropion and hydroxybupropion in human plasma and urine by LC/MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; **857**(1): 67–75.

18. Laizure SC, DeVane CL, Stewart JT, Dommisse CS, Lai AA. Pharmacokinetics of bupropion and its major basic metabolites in normal subjects after a single dose. *Clin Pharmacol Ther* 1985; **38**(5): 586–589.

19. Hesse LM, He P, Krishnaswamy S, *et al.* Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes. *Pharmacogenetics* 2004; **14**(4): 225–238.

20. GlaxoSmithKline. *Wellbutrin (bupropion hydrochloride) Tablets.*

21. Gervot L, Rochat B, Gautier JC, *et al.* Human CYP2B6: expression, inducibility and catalytic activities. *Pharmacogenetics* 1999; **9**(3): 295–306.

22. Oppermann U. Carbonyl reductases: the complex relationships of mammalian carbonyl- and quinone-reducing enzymes and their role in physiology. *Annu Rev Pharmacol Toxicol* 2007; **47**: 293–322.

23. Taylor EW, Jia W, Bush M, Dollinger GD. Accelerating the drug optimization process: identification, structure elucidation, and quantification of *in vivo* metabolites using stable isotopes with LC/MSn and the chemiluminescent nitrogen detector. *Anal Chem* 2002; **74**(13): 3232–3238.

24. Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* 2003; **43**: 149–173.

25. Faucette SR, Hawke RL, Lecluyse EL, *et al.* Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab Dispos* 2000; **28**(10): 1222–1230.

26. Janmohamed A, Dolphin CT, Phillips IR, Shephard EA. Quantification and cellular localization of expression in human skin of genes encoding flavin-containing monooxygenases and cytochromes P450. *Biochem Pharmacol* 2001; **62**(6): 777–786.

27. Wang H, Tompkins LM. CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr Drug Metab* 2008; **9**(7): 598–610.

28. Oppermann UC, Maser E. Molecular and structural aspects of xenobiotic carbonyl metabolizing enzymes. Role of reductases and dehydrogenases in xenobiotic phase I reactions. *Toxicology* 2000; **144**(1–3): 71–81.

29. Penning TM, Drury JE. Human aldo-keto reductases: function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys* 2007; **464**(2): 241–250.

30. Court MH. Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. *Drug Metab Rev* 2010; **42**(1): 209–224.

31. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 2003; **3**(3): 136–158.

32. Chen Y, Liu HF, Liu L, Nguyen K, Jones EB, Fretland AJ. The *in vitro* metabolism of bupropion revisited: concentration dependent involvement of cytochrome P450 2C19. *Xenobiotica* 2010; **40**(8): 536–546.

33. Faucette SR, Hawke RL, Shord SS, Lecluyse EL, Lindley CM. Evaluation of the contribution of cytochrome P450 3A4 to human liver microsomal bupropion hydroxylation. *Drug Metab Dispos* 2001; **29**(8): 1123–1129.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Figure S1. Isotopic distribution of metabolites newly found in human plasma. (A) M2 ($m/z = 432$), (B) M3 ($m/z = 258$) and (C) M4 ($m/z = 418$). M4 to M7 have the same isotopic pattern