Case Report

A Fatal Case of Bupropion (Zyban®) Overdose

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Abstract

A sensitive and reproducible method for the identification and the quantitative determination of bupropion (BUP) and its major metabolites, hydroxybupropion (OH-BUP) and threohydrobupropion (T-BUP), was developed in blood and urine. The three compounds were extracted with a solid-phase extraction procedure followed by LC–ESI-MS–MS separation and quantification using decadeuterated lidocaine as internal standard. BUP and its metabolites were satisfactorily identified by multiple reactions monitoring detection. The limits of detection and quantification were determined at 5 and 10 µg/L, respectively, for each analyte. The intraday and interday coefficients of variability were lower than 11.9% for BUP and its metabolites. This method was applied to the forensic case of a 35-year-old male who died after a suspected ingestion of 30 slow-release tablets of Zyban. As samplings were performed at least 72 h after the drug intake, BUP had disappeared from blood, but OH-BUP and T-BUP were present at the concentrations of 5.8 and 30.4 mg/L, respectively. In urine, concentrations ranged from 42.9 mg/L for BUP to 617 mg/L for T-BUP. These results agree with the hypothesis of a successful suicide attempt.

Introduction

Tobacco use remains one of the major causes of death in the world. For many years, several European countries have launched active campaigns against tobacco use. Several nicotine substitution products have been proposed to smokers in order to help them to stop. In 1997, the Food and Drug Administration (FDA) approved the use of an antidepressant drug, bupropion (BUP), to help adult smokers to stop tobacco use (1).

Originally approved for the treatment of depression in 1985, BUP was removed from International Pharmacopeia one year later because of the important risk of seizures, mainly identified in sub-populations with epilepsy or a history of head trauma (2). Complementary studies showed that, at a lower dose range, the incidence of seizures was similar to that of other antidepressant drugs. The product was reintroduced in 1989 with special attention paid to side effects in patients with known epilepsy or suffering from eating disorders (1,2). The daily oral dose, used for depression as well as smoking cessation, is about 300 mg, administered in normal release tablets of 75 or 100 mg (depression) or in sustained release tablets of 150 mg (smoking cessation). Therapeutic concentrations of BUP in blood when these doses are used range between 25 and 100 µg/L (3).

BUP is a monocyclic antidepressant, chemically unrelated to tricyclic, tetracyclic, or other known antidepressant agents. This drug is structurally similar to amphetamine and diethylpropion, an anorexigenic drug. Its therapeutic efficacy is, however, comparable to that of classical antidepressants. In fact, bupropion is a dual dopaminergic and noradrenergic reuptake inhibitor, presenting weak but relatively selective inhibition characteristics of dopamine reuptake (4).

In smoking cessation, BUP does not replace nicotine, but it is believed to act by increasing dopamine levels in the *nucleus accumbens* because dopamine level can be decreased during cigarette abstinence (5).

After two or three years of prescription for tobacco-use cessation, several articles concerning bupropion toxicity appeared in the literature (6–8). The clinical toxicity of BUP overdose included sinusoidal tachycardia, hypertension, hallucinations, agitation, seizures and tremors, and sometimes drowsiness. These side effects might be prolonged with the sustained-release forms of bupropion (Zyban, Wellbutrin SR®) (9).

Cardiac manifestations are uncommon (except for tachycardia); nevertheless, overdose with more than 1.5 g has been associated with disturbance of intraventricular conduction and prolongation of the QT interval. With massive overdose (10 g or more), cardiac failure and death can occur in absence of fast treatment. The rates of 0.01 and 0.11 deaths per 1000 prescriptions, respectively, were reported in Canada and the United Kingdom. Almost all deaths were related to intentional BUP overdoses (10).

Very few postmortem toxicological data including BUP have been published (11–13). The interpretation of blood concentrations is complicated by the fact that BUP is unstable in blood and is extensively metabolized by multiple pathways into two major and one minor active metabolites, hydroxybupropion (OH-BUP) and the amino-alcohol isomers, threohy-

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drobupropion (T-BUP) and erythrohydrobupropion (E-BUP). The identification and quantification of these metabolites are essential to reveal intoxications by BUP (14,15). Figure 1 presents chemical structures of BUP, OH-BUP, T-BUP, and E-BUP.

The present article describes a liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS–MS) method for the rapid and sensitive identification and quantification of BUP and its metabolites in blood and urine and its application to a fatal case of Zyban overdose.

Case History

A 35-year-old male was found lying in his bed about 72 h after he died. An empty box of Zyban (30 slow-release tablets of 150 mg of BUP) was near the body. The young man had been following a six-month treatment to stop tobacco use. No other known medication was involved. No goodbye letter was found in the room, but he had become increasingly depressed according to his family. The incident occurred during a very hot period in the summer, which led to the partial decomposition of the body. No autopsy was imposed by legal authorities, so only femoral whole blood and urine samples were collected for analysis during the external examination.

Materials and Methods

Chemicals and reagents

BUP, OH-BUP, and T-BUP were kindly provided by GlaxoSmithKline (Stevenage, U.K.) as hydrochloride salts. The internal standard (I.S.), decadeuterated lidocaine, was obtained from C/D/N Isotopes (Pointe-Claire, QC, Canada). Methanol and acetonitrile were purchased from Carlo Erba Reagents (Val de Reuil, France); formic acid (99% min) and ammonium formate were from Sigma (Saint Quentin Fallavier, France); and methylene chloride, propan-2-ol, and zinc sulfate were from VWR (Strasbourg, France).

Preparation of standard solutions

BUP, OH-BUP, T-BUP, and I.S. powders were separately dissolved in methanol to obtain stock solutions at 1 g/L. Then the solutions were mixed and appropriately diluted in deionized water to obtain four pools of working solutions of BUP, OH-BUP, and T-BUP at 5, 1, 0.5, and 0.1 mg/L, and the I.S. was diluted in deionized water at 5 mg/L. These standard solutions were stored at +4°C.

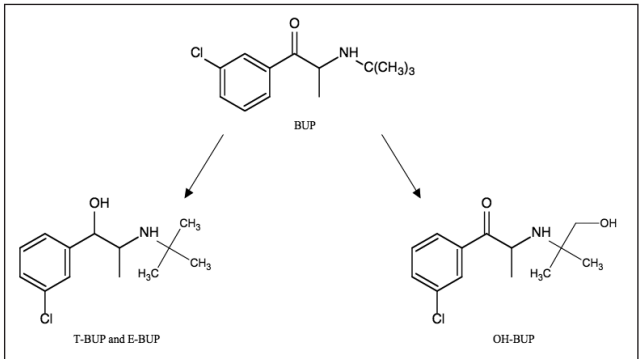

Figure 1. Chemical structures of BUP, OH-BUP, T-BUP, and E-BUP.

Instrumentation

The chromatographic system consisted of a series 200LC micro-flow rate, high-pressure gradient pumping system (Perkin-Elmer Instruments, Les Ulis, France) including a Rheodyne model 7725 injection valve equipped with a 20-µL internal loop. Two mobile phases previously degassed by nitrogen were employed. Mobile phase A was ammonium formate buffer (2mM, pH 4.0). Mobile phase B was a 90:10 (v/v) mixture of acetonitrile and ammonium formate buffer (2mM, pH 4.0). The flow rate was 50 µL/min. A SymmetryShield RP 18 column (150 × 1.0-mm i.d., 3.5 µm, Waters, Milford, MA) was used for compound separation with a gradient of mobile phases A and B programmed as following: 0–1.0 min, 5% B; 1.0–1.5 min, 5 to 35% B; 5.0–5.5 min, 35 to 40% B; 5.5–7.5 min, 40 to 95% B; 7.5–8.5 min, decrease from 95 to 5% B; and 8.5–12 min, column equilibration with 5% B.

Detection was carried out with an API 2000 LC–MS–MS System (Applied Biosystems/MDS Sciex, Concord, ON, Canada) equipped with a TurboIonSpray ionization source and controlled by Analyst® software. The TurboIonSpray settings were optimized by infusing, at 5 µL/min, a 1 mg/L solution of the four compounds (BUP, OH-BUP, T-BUP, and I.S.) prepared in a 30:70 (v/v) mixture of acetonitrile and formate buffer (2mM, pH 4.0). In the positive mode, the optimal settings of the ionization in the source were ion spray voltage at 5000V and curtain gas and ion source gas 1 at 40 and 20 units, respectively. The declustering potential (DP) was optimized for each compound as shown in Table I.

Sample preparation

Calibration standards at 0, 10, 25, 50, 100, 200, and 500 µg/L were prepared by spiking 100 µL of drug-free human whole blood and urine with 10 µL of the I.S. solution prepared at 5 mg/L and the appropriate volumes of the different working solutions.

Table I. Optimized Mass Spectrometric Parameters

Analytes	Q1 Mass* (amu)	Q3 Mass (amu)	DP (V)	FP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)
I.S.	246.0	96.2	41.0	360.0	–10.0	12.0	27.0	0.0
OH-BUP	255.9	238.0	11.0	360.0	–6.0	14.0	15.0	4.0
	255.9	167.1	16.0	350.0	–6.5	20.0	33.0	0.0
BUP	239.9	166.0	16.0	370.0	–6.5	14.0	25.0	2.0
	239.9	184.0	16.0	370.0	-6.0	12.0	17.0	2.0
T-BUP	242.3	168.2	16.0	360.0	–9.5	14.0	23.0	0.0
	242.3	186.0	16.0	360.0	–10.5	14.0	15.0	2.0

* Q1 mass, parent ion mass-to-charge ratio; Q2 mass, daughter ion mass-to-charge ratio; DP, declustering potential (orifice plate); FP, focusing potential (focusing ring); EP, entrance potential (Q0 less); CEP, cell entrance potential; CE, collision cell potential; and CXP, cell exit potential.

Then 0.5 mL of a 70:30 (v/v) mixture of zinc sulfate saturated aqueous solution and methanol was added to blood (not to urine) in order to precipitate blood proteins. Spiked blood and urine samples were vortex mixed for 10 s and centrifuged at 3000 rpm for 5 min. Supernatants were transferred to 15-mL glass tubes, added to 2 mL of deionized water, and vortex mixed for 10 s. Oasis® HLB extraction cartridges were conditioned by 2 mL of methanol and equilibrated by 2 mL of deionized water. Then supernatants were loaded, and cartridges were successively washed by 3 mL of deionized water and 3 mL of a 90:10 (v/v) mixture of deionized water and methanol. Cartridges were dried for 15 min, and washing solutions were eliminated. Solutes were eluted by 3 mL of a methylene chloride and propan-2-ol (75:25, v/v) mixture and collected in 10-mL glass tubes. Eluates were evaporated under nitrogen flux at room temperature and reconstituted with 50 µL of a 10:90 (v/v) mixture of acetonitrile and formate buffer (2mM, pH 4.0). These extracts were introduced in 200-µL vials for injection, and 10 µL was injected into our LC–ESI-MS–MS system (16).

Results and Discussion

BUP and its main metabolites were identified by multiple reactions monitoring (MRM) detection. Table II shows the mass transitions of each analyte and their retention times. The "mass transition 1" corresponds to the mass transition used for the quantification; the "mass transition 2" confirms the first one.

Table II. Mass Transitions, Retention Times, and Relative Retention Times of I.S., BUP, OH-BUP, and T-BUP

Analytes	Mass Transition 1 (*m/z* > *m/z*)	Mass Transition 2 (*m/z* > *m/z*)	Retention Time (min)	Relative Retention Time
I.S.	246.0 > 96.2	–	6.76	–
OH-BUP	255.9 > 238.0	255.9 > 167.1	6.94	1.03
BUP	239.9 > 166.0	239.9 > 184.0	7.14	1.06
T-BUP	242.3 > 168.2	242.3 > 186.0	7.14	1.06

The particular case of the amino-alcohol isomers, T-BUP and E-BUP, was studied. Indeed, T-BUP and E-BUP co-elute (relative retention time: 1.06) and show the same MRM transitions with equivalent abundances under our LC–ESI-MS–MS conditions. Moreover, previous pharmacokinetics studies have shown that E-BUP concentrations in human plasma are very low and only contribute to a small portion of the overall combination peak (17–22). As a consequence, we decided to analyze and quantify the main analyte, T-BUP.

Method validation

Calibration curves obtained between 10 and 500 µg/L for BUP and its two main metabolites, using linear regression without weighting, gave excellent correlation coefficients (r > 0.993). For all analytes, the limits of detection and quantitation were 5 and 10 µg/L, respectively.

Extraction recovery was determined by comparing the representative peak areas of extracted drug-free blood (or urine) spiked before extraction, with the peak area of drug-free blood (or urine) fortified before injection, at the same concentration. The extraction recoveries were higher than 54.3%, 63.4%, and 60.5%, respectively, for BUP, OH-BUP, and T-BUP. Coefficient of variability (C.V.) values were determined at 10, 50, 200, and 500 µg/L by replicate analyses (n = 5) of human whole blood and urine aliquots, either on the same run (intraday) or on separate days (interday) and proved that the method is precise (intraday and interday C.V. were lower than 11.9% for BUP, OH-BUP, and T-BUP). Method validation data have been detailed in Table III.

Matrix ion suppression

Matrix ion suppression effects on the MRM LC–MS–MS sen-

Table III. Method Validation Data in Blood

Products	Spiked Concentrations (µg/L)	*n**	Within-Day CV (%)	Within-Day Accuracy (%)	Between-Day CV (%)	Between-Day Accuracy (%)	Measurement Uncertainty (%)	Extraction Recovery (%)	Extraction Recovery CV (%)
BUP	10	5	3.8	4.1	9.8	2.9	23.0	79.3	16.7
	50	5	11.0	–7.2	7.6	–2.3	17.2	64.6	11.0
	200	5	7.6	1.0	4.0	–8.6	16.0	54.3	7.7
	500	5	11.9	16.3	8.5	–9.3	24.8	70.4	11.9
T-BUP	10	5	4.0	1.0	3.7	6.8	14.7	61.2	17.4
	50	5	3.2	9.2	5.5	1.5	12.7	70.1	3.1
	200	5	3.3	0.7	5.6	2.4	13.8	60.5	3.5
	500	5	4.7	3.4	3.3	2.1	8.8	64.5	4.6
OH-BUP	10	5	3.1	–1.1	1.5	3.0	6.2	63.4	6.4
	50	5	2.0	4.0	4.3	4.6	13.6	90.0	1.8
	200	5	2.2	–5.1	4.5	1.7	10.9	73.0	2.2
	500	5	2.2	–1.2	3.7	5.8	13.6	75.9	2.3

* *n*, number of determinations and CV%, coefficient of variation.

sitivity were evaluated by the experiment of the post-column analyte infusion described by Antignac et al. (23). A standard solution containing BUP, OH-BUP, and T-BUP at 100 µg/L and I.S. at 500 µg/L in mobile phase was infused post-column via a T-shaped connector, at 50 µL/min, using an infusion pump. Ten different blank (no spiked) whole blood and urine samples were extracted. Ten microliters of extracted bloods and urines was then injected onto an HPLC column at a 50 µL/min mobile phase flow by a Perkin Elmer autosampler, and MRM LC–MS–MS chromatograms were acquired for each analyte. No significant ion suppression effect was observed because of matrix components present in reconstituted extracts.

Case report

Comprehensive toxicology testing was performed. The qualitative drug screen, a combination of fluorescence polarization immunoassay and gas and liquid chromatography of the blood and urine, showed the presence of bupropion and its metabolites. No other drugs or ethyl alcohol were found. The results of the toxicological analysis of our reported fatal case are presented in Table IV. No BUP was found in the femoral blood, and OH-BUP and T-BUP were detected and respectively quantified at 5.8 and 30.4 mg/L after suitable dilutions were made. Figure 2 shows chromatograms of the peripheral blood of our reported fatal case. In urine, BUP and its active metabolites were detected at very high concentrations: BUP at 42.9 mg/L, OH-BUP at 100 mg/L, and T-BUP at 617.0 mg/L.

Table IV. Concentrations of BUP and its Metabolites Obtained after Suitable Dilution of the Postmortem Samples of the Reported Fatal Case

Sample	BUP (mg/L)	OH-BUP (mg/L)	T-BUP (mg/L)
Femoral blood	Not detected	5.8	30.4
Urine	42.9	100	617

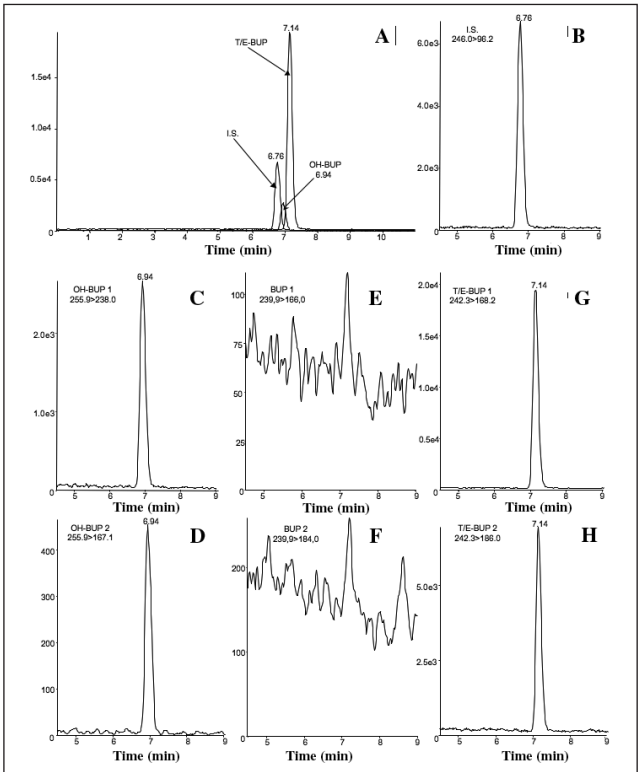

Figure 2. Chromatograms of the peripheral blood (dilution: 1:100) of our reported fatal case spiked with I.S.: full chromatogram (A); I.S. mass transition (B); OH-BUP mass transitions 1 and 2 (C, D); BUP mass transitions 1 and 2 (E, F); and T/E-BUP mass transitions 1 and 2 (G, H).

In this case study, bupropion could be responsible for death, even though there was no BUP in the blood at the time of the analysis. The femoral blood concentration levels of OH-BUP and T-BUP, 5.8 and 30.4 mg/L, respectively, seem to be within the range of lethal concentrations in postmortem blood. Friel et al. (13) studied three fatal overdoses involving BUP and presented peripheral blood levels as high as 4.0, 3.4, and 11.5 mg/L, respectively, for BUP, OH-BUP, and T-BUP.

To date, if the symptomatology of intoxication by BUP is well documented, there are few references to blood concentrations of BUP and its metabolites in cases of lethal intoxication by this drug (11,13,15).

In this fatal case, the femoral blood sample was taken about 72 h after the death and refrigerated 4 days before the testing was done. The analysis did not reveal the presence of BUP. This result was not surprising insofar as Laizure and DeVane (14) demonstrated that about 50% of the active compound BUP was damaged in plasmatic samples stocked at 22°C and the totality at 37°C, for more than 48 h. Moreover, the authors also explain that BUP does not degrade into any of the three metabolites, OH-BUP, E-BUP, and T-BUP. As a consequence BUP was probably damaged by the hydrolysis of the carbon-nitrogen bond of the amino group. The studies of Laizure and DeVane (14) demonstrated on the other hand the very good stability of these three metabolites. Therefore, we can suppose that the blood concentrations of OH-BUP (5.8 mg/L) and T-BUP (30.4 mg/L) determined with samples taken three days after the death are in accordance with antemortem concentrations, without taking redistribution postmortem into account. On this assumption, these concentrations are quite comparable with a lethal overdose observed by other authors: from 1.7 to 5.0 mg/L for OH-BUP and from 4.6 to 17.8 mg/L for T-BUP (11,13,15).

In blood, lethal concentrations are much higher than therapeutic concentrations (therapeutic concentrations of BUP in blood ranged between 25 and 100 µg/L) (3). Considering toxicological findings (lethal blood concentrations of metabolites) and the features of BUP toxicity symptoms in case of overdose described by Jepsen et al. (9) (cardiovascular with prolonged QRS and QT, and neurological with seizure and coma), the death could be the result of BUP toxic effects, although the death circumstances were unknown.

Conclusions

We developed a specific and sensitive LC–ESI-MS–MS method for the identification and the quantification of BUP and its main metabolites in blood and urine. This method was validated from 10 to 500 µg/L for each analyte. Applied to a forensic case of a suicide with BUP, the method revealed lethal

concentrations in blood (only metabolites were found because BUP is unstable) and urine. This sensitive method seems to be able to monitor bupropion in tobacco substitution, as well as to reveal intoxications by bupropion in forensic cases when Zyban is implicated (plasmatic therapeutic concentrations of BUP, OH-BUP, and T-BUP range between 20 and 100 µg/L, 94 and 486 µg/L, and 27 and 213 µg/L, respectively) (3,24). In this forensic case, BUP had disappeared from blood but was identified in urine at very high concentration, and OH-BUP and T-BUP were detected and quantified in blood and urine at lethal or at least very high concentrations. BUP seemed to be responsible of death because no other drug neither drug of abuse was revealed by general blood and urine screening.

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