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The development and validation of a turbulent flow-liquid chromatography–tandem mass spectrometric method for the simultaneous quantification of citalopram, sertraline, bupropion and hydroxybupropion in serum

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ABSTRACT

Objectives: Depression is a rapidly growing issue in the United States. There are many drug classes that may be used to treat depression, including the selective serotonin-reuptake inhibitors (SSRIs) citalopram (Celexa®) and sertraline (Zoloft®), as well as the aminoketone bupropion (Wellbutrin®). However, therapeutic efficacy and treatment success is often variable, requiring changes in dosing regimens or drug selection. Methods for drug quantification can become important tools in the assessment of drug efficacy to optimize treatment regimens. Here, we present a turbulent flow-liquid chromatography–tandem mass spectrometric (TFC–MS/MS) method for the robust, simultaneous quantification of citalopram, sertraline, bupropion and its active metabolite, hydroxybupropion (OH-bupropion).

Design and methods: Serum spiked with the aforementioned antidepressants, along with their corresponding isotopically labeled internal standards was subjected to protein precipitation. Samples were injected onto a TFC column for on-line solid phase extraction and a Hypersil Gold C18 column for chromatographic separation. Detection was achieved using a TSQ Vantage mass spectrometer. Assay validation followed FDA bioanalytical guidelines.

Results: The analytical measuring range for all analytes spanned from 5 to 1000 ng/mL. Intra- and inter-assay precision across four quality control levels were ≤9.2% and ≤14.8%, respectively. A comparison to other LC–MS/MS methods resulted in a strong correlation with correlation coefficients ranging from 0.9929 to 0.9971. Carryover, stability, recovery, matrix effects, extraction and processing efficiency were also deemed acceptable in accordance with FDA recommendations.

Conclusions: The development and validation of this TFC–MS/MS method allow for the robust and high-throughput quantification of commonly prescribed antidepressants.

Introduction

Depression disorders affect approximately 10% of the American population and are recognized via a constellation of symptoms, including depressed mood, changes in appetite or weight, sleep disturbances, and psychomotor abnormalities [1,2]. Depressive disorders may be classified as major depressive, subsyndromal or chronic. A primary treatment modality for depression is antidepressant administration to control symptoms and prevent relapses [2]. Antidepressant drugs, including the non-tricyclic aminoketone bupropion (marketed as Wellbutrin®), have been used since the 1980s in disease management. More recently, second-generation antidepressants, including selective serotonin reuptake inhibitors (SSRIs) such as citalopram (Celexa®), escitalopram (Lexapro®), and sertraline (Zoloft®), have been employed for symptom mitigation [3,4]. Notably, citalopram and sertraline have also been used in the successful treatment of anxiety, panic, post-traumatic stress and obsessive–compulsive disorders [2].

The described antidepressants exhibit high pharmacokinetic variability, chiefly due to adherence and compliance; however, genetic variations in metabolizing enzymes, environmental factors and clinical conditions, also impact therapeutic efficacy [5]. Further, pharmacotherapeutic

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success is highly dependent on drug dose and selection, which can be frequently altered, until the patient's condition shows signs of improvement. Therapeutic ranges have been suggested by the AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry at 55–110 ng/mL for citalopram, 10–150 ng/mL for sertraline, and 225–1500 ng/mL for both bupropion and hydroxybupropion (OH-bupropion) [6]. Toxicity associated with SSRIs is uncommon, but adverse events including tachycardia, tremor, drowsiness, nausea and vomiting have been reported [7–10]; rarely, high doses of SSRIs can cause seizures, cardiotoxicity, and death at drug concentrations ranging from of 0.8 to 16 mg/L [9–13]. Therefore, assessing blood concentrations of these agents may be important in directing dosage adjustments and in the evaluation of subclinical or toxic effects [6].

Therapeutic drug monitoring (TDM) has proven to be a valuable tool in the field of psychiatry, especially in understanding the efficacy of lithium, tricyclic antidepressants, and mood stabilizing drugs [14,15]. Various methods have been described for the simultaneous quantification of sertraline and citalopram using gas-chromatography–mass spectrometry (GC–MS) [16], high performance liquid chromatography–ultraviolet detection (HPLC–UV) [17–19], or HPLC–tandem mass spectrometry (HPLC–MS/MS) [20–24]. Many of the previously reported studies do not include isotopically labeled internal standards, or involve significant sample pre-treatment prior to analysis [18,19,23,24]. Methods for bupropion and OH-bupropion have also been published, but do not include the simultaneous quantification of the aforementioned SSRIs [25–27]. Here we present a turbulent flow liquid chromatography–tandem mass spectrometric (TFC–MS/MS) method for the quantification of citalopram, sertraline, and bupropion, as well as the bupropion metabolite OH-bupropion. The inclusion of these antidepressant drugs in one panel over a wide analytical measuring range (5–1000 ng/mL) allows for the monitoring of a diverse group of individuals.

Methods

Chemicals

Stock solutions of citalopram, sertraline, bupropion, OH-bupropion, as well as the isotopically labeled internal standards (IS) citalopram-D6, sertraline-D3, bupropion-D9, and OH-bupropion-D6, were all purchased from Cerilliant Corporation (Round Rock, TX) (Fig. 1). Drug-free human serum was purchased from BioRad Laboratories (Irvine, CA). Formic acid was acquired from Sigma (St. Louis, MO), and acetonitrile, HPLC-grade water, and methanol were purchased from Fisher Scientific (Pittsburgh, PA). For selectivity and matrix effects studies, remnant human serum was acquired from vacutainer serum separator tubes (SST) (BD, Franklin Lakes, NJ) via an Institutional Review Board (IRB)-approved protocol through the Johns Hopkins University School of Medicine.

Preparation of reagents and standards

All stock solutions were diluted in methanol (MeOH) to achieve working stock solutions containing the aforementioned analytes at concentrations of 0.1, 1, 10, and 100 μg/mL. Calibration standards for all analytes were prepared in drug free serum at final drug concentrations of 5, 10, 50, 100, 150, 250, 500, 750, and 1000 ng/mL. Further, using independent working stock solutions, quality control (QC) samples were prepared at the lower limit of quantification (LLOQ), as well as low, mid and high levels. LLOQ, low, mid and high QC levels contained 5, 15, 125 and 850 ng/mL mixtures of the aforementioned drugs, respectively. Analytes were isolated via protein precipitation, in which 100 μL of specimen was combined with 300 μL of an acetonitrile mixture containing the aforementioned IS mixture at a final concentration of 40 ng/mL for each standard. Specimens were vortexed for 30 s and centrifuged at 12,000 rpm for 5 min at room temperature; 300 μL of the supernatant was then diluted 1:1 with water, and subjected to further online solid phase extraction (SPE) and chromatographic separation on the Prelude turbulent flow SPLC system (Thermo Scientific, San Jose, CA).

Liquid chromatography conditions

The Prelude TFC system (Thermo Scientific, San Jose, CA) was comprised of an Aria TLX1 system equipped with 1250 transcend pumps and a CTC PAL autosampler with a sample stack, which was maintained at 4 °C. The TLX1 system utilizes a six-port switching valve, allowing for online sample extraction, which was conducted using a Cyclone-P (0.5 × 50 mm) column (Thermo Scientific, Pittsburgh, PA).

Compounds were chromatographically separated using a Hypersil Gold C18 (50 × 2.1 mm, 1.9 μm particle size) column that was maintained at 40 °C. A mobile phase system comprised of water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) was used. The four analytes and their respective internal standards were eluted under a solvent gradient from 30% to 60% mobile phase B. Once elution was completed, the column was washed with 95% mobile phase B for 1 min and subsequently re-equilibrated to 100% mobile phase A for 1 min. The total analytical run time for this assay was 5 min.

Mass spectrometric parameters

Antidepressant monitoring was achieved using a TSQ Vantage tandem mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a heated electrospray ionization source (HESI). Optimization of mass spectrometric conditions was achieved by direct infusion of each of the four analytes at a flow rate of 10 μL/min into the mass analyzer. The instrument was operated in selective reaction monitoring (SRM)

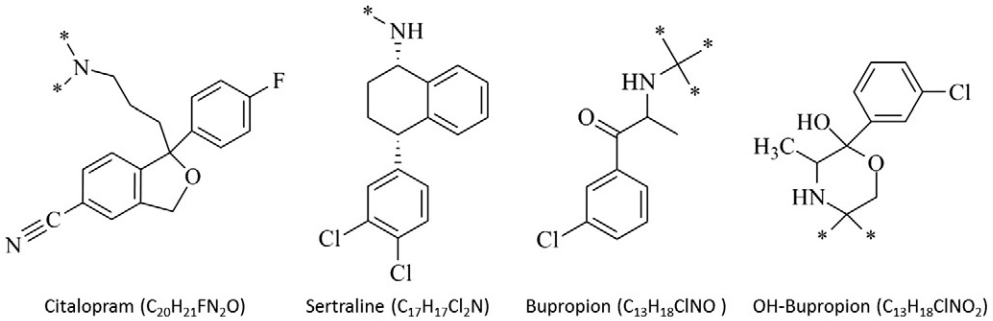

Fig. 1. Chemical structures of monitored antidepressants. Citalopram (CIT), sertraline (SER), buproprion (BUP) and its active metabolite hydroxybupropion (OH-BUP) are depicted. The chemical formula and molecular weight are denoted below each structure.