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Simultaneous determination of bentazone and its metabolites in postmortem whole blood using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A liquid chromatography–tandem mass spectrometry method with solid-phase extraction (SPE) was developed and validated for the detection and quantitation of bentazone and its two hydroxylated metabolites, 6-hydroxybentazone and 8-hydroxybentazone, in postmortem blood. Sample cleanup was performed using a hydrophilic-lipophilic balanced (HLB) SPE cartridge and then separated on a C18 LC column using a gradient elution of 0.1% formic acid in distilled water and 0.1% formic acid in methanol. The identification of bentazone and its hydroxylated metabolites was performed using tandem mass spectrometry with electrospray ionization in negative ion mode with selective reaction monitoring. The retention times of bentazone, 6-hydroxybentazone, 8-hydroxybentazone, and 2-methyl-4-chlorophenoxyacetic acid (MCPA, internal standard) appeared separately in the chromatogram. The matrix effect, recovery, and process efficiency of bentazone were 75.3%, 103.6% and 77.9%, respectively. In addition, good accuracy (88.2–110.5%), precision (0.5–7.5%, bias), and linearity (5–500 ng/mL) were obtained with this method. The limit of detection (LOD) of bentazone, 6-hydroxybentazone, and 8-hydroxybentazone were 0.05, 0.5, and 0.5 ng/mL, respectively. The method developed herein was applied to authentic samples from three fatal cases from 2016 for the determination of the corresponding bentazone and its metabolites levels. The concentration ranges of bentazone, 6-hydroxybentazone, and 8-hydroxybentazone in the heart blood from the three victims were 46.0–91.8, 4.2–6.2, and 0.2–0.6 μg/mL, respectively.

1. Introduction

Initially registered in 1975, bentazone (3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) is a herbicide that has been used under the trade name Bentazone® and Basagran® [1]. Bentazone is used for the selective control of broadleaf weeds and sedges in beans, rice, corn, peanuts, and mint by suppressing the plant's ability to use sunlight for photosynthesis, which is vital for energy production and hence survival. Visible injury to treated leaf surfaces usually occurs within 4–8 h, which is followed by plant death [1,2].

Bentazone has been considered moderately toxic upon ingestion and slightly toxic upon dermal absorption in mammals, and WHO has classified bentazone as slightly hazardous [3]. The acute oral toxicity (LD_{50}) is 400 mg/kg in mice, 750 mg/kg in rabbits, and 1100 mg/kg in rats [1,2], but its toxicity in humans is not clearly known. The reported toxic symptoms of bentazone ingestion include vomiting, diarrhea, trembling, weakness, irregular or difficult breathing [4,5]. Large ingested doses of bentazone have led to acute hepatitis, acute renal failure, and even death [5].

Despite these toxic symptoms from exposure to bentazone, few studies have been reported on its effects following human ingestion. Lin et al. reported an acute case of BASAGRAN® (sodium salt of bentazone, 44.0%) poisoning in a patient who ingested approximately 130 g of bentazone for the purpose of suicide [6]. In this case, vomiting, sweating, fever, muscle rigidity, leukocytosis, renal damage, and rhabdomyolysis appeared. However, an analysis of the victim's blood or plasma to measure the associated bentazone concentrations was not performed. Turcant et al. reported a fatal case of bentazone ingestion of a 56-year-old farmer who voluntarily ingested 500 mL of FIGHTER® (bentazone, 480 g/L in water); the reported symptoms included vomiting, diarrhea, difficulty in breathing, muscle rigidity, and finally cardiac arrest [7]. In this case, the plasma concentration of bentazone was 1500 μg/mL. Jung et al. reported the case of a 54-year-old female who was brought to the hospital 1.5 h after ingesting 168 g of bentazone (3055 mg/kg) [8]. The woman suffered several toxic symptoms including vomiting, diarrhea, fever, and

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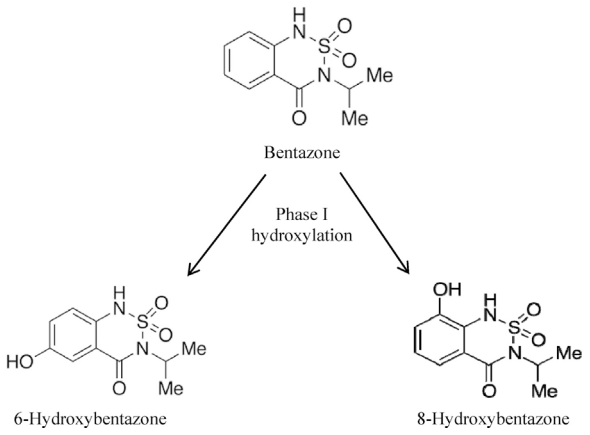

Fig. 1. Chemical structures of bentazone, 6-hydroxybentazone, and 8-hydroxybentazone.

rhabdomyolysis. However, the concentration of bentazone in the postmortem blood was not measured.

Several analytical methods have been reported in the literature to detect and measure bentazone in water, including HPLC, UV/VIS spectroscopy, gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [9–11]. Although, 6-hydroxybentazone and 8-hydroxybentazone are known as phase I metabolites of bentazone (Fig. 1) [5,7,10], there are very few reports on the analysis of bentazone and its major metabolites in biological samples. Thus, it is necessary to establish and validate an analytical method for the accurate determination of bentazone and its metabolites in biological samples.

To this end, this study tested several solid-phase extraction (SPE) cartridges to select the optimal conditions for sample preparation, followed by LC–MS/MS analysis of bentazone and its two major metabolites (Fig. 1). The developed method was applied to authentic blood samples derived from recent bentazone-related fatal deaths for the identification and quantitation of bentazone and its major metabolites, 6-hydroxybentazone and 8-hydroxybentazone.

2. Materials and methods

2.1. Reagents and materials

A solution of bentazone (100 μg/mL) was purchased from Kemidas Co., Ltd. (Suwon, Korea), 6-hydroxybentazone powder (97%) was purchased from Toronto Research Chemical Inc. (Toronto, Canada), and a solution of 8-hydroxybentazone (100 μg/mL) was purchased from HPC Standard GmbH (Cunnersdorf, Germany). 2-Methyl-4-chlorophenoxyacetic acid (MCPA) was purchased from Sigma Aldrich (St. Louis, MO, USA). A hydrophilic-lipophilic balanced (HLB) SPE cartridge (3 mL) was purchased from Waters Corporation (Milford, USA). HPLC grade methanol and acetonitrile were purchased from Fischer Scientific Co. (Fair Lawn, NJ, USA). Distilled water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals were the highest quality available and used without further purification.

2.2. Instruments

2.2.1. GC–MS

The GC–MS system consisted of an Agilent 7980A gas chromatograph with a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and an Agilent 5975MSD mass spectrometer (CA, USA). The injection port temperature was set to 260 °C. The oven temperature was held at 80 °C for 1 min, elevated to 180 °C at 20 °C/min and then elevated to 300 °C at 30 °C/min, where it was held for 10 min, giving a total run time of 30 min. A 1 μL aliquot was injected into the GC in splitless mode. The mass spectrometer was run in electron ionization (EI) mode with 70 eV of energy.

2.2.2. LC–MS/MS

The HPLC system consisted of an Agilent 1290 with a binary pump, a degasser, a thermo-stated autosampler, and a column oven compartment (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was performed in a Cadenza CD-C18 column (3 μm, 100 mm × 2.0 mm i.d.; Imtakt Corporation, PA, USA). The mobile phase was composed of 0.1% formic acid in distilled water and 0.1% formic acid in methanol, and delivered at 0.3 mL/min in gradient mode.

The blood concentrations of bentazone and 6- and 8-hydroxybentazone were quantified using an AB SCIEX 4500 QTRAP mass spectrometer equipped with a Turbo Ion Spray to generate the negative ions $[M-H]^-$. The mass fragments of the precursor ions for quantification of bentazone and its metabolites were determined in multiple reaction monitoring (MRM) mode using the Analyst® software. The MRM transitions were selected as follows (Table 1): m/z 238.6 → 131.8, 196.9 for bentazone; m/z 254.9 → 147.9, 190.8 for 6-hydroxybentazone; m/z 254.9 → 190.8, 147.9 for 8-hydroxybentazone; and m/z 198.8 → 140.8 for MCPA (internal standard).

2.2.3. LC–TOF/MS

The HPLC system consisted of an Shiseido NANOSPACE SI-2 (Shiseido, Japan) with a degasser, a thermo-stated autosampler, and a column oven compartment. Chromatographic separation was performed in a Kinetex F5 column (2.6 μm, 100 mm × 2.1 mm i.d.; Phenomenex, USA). The mobile phase was composed of 0.1% formic acid in distilled water and 0.1% formic acid in methanol, and delivered at 0.3 mL/min in gradient mode.

For the mass spectrometer, AB SCIEX's Triple TOF™ 6600 mass spectrometer equipped with a Turbo Ion Spray was used in electrospray ionization (ESI) negative ions $[M-H]^-$ mode. Analyst® software package was used in instrument settings, data acquisitions and processing.

2.3. Sample preparation

2.3.1. Standard stock solutions

Standard stock solutions of 100 μg/mL of bentazone and 6- and 8-hydroxybentazone were dissolved in acetonitrile and stored at −20 °C in a refrigerator until analysis. These were serially diluted with acetonitrile to prepare 10, 5, 2, 1, 0.2, and 0.05 μg/mL working standard solutions. The MCPA stock solution was diluted with methanol to a concentration of 0.1 μg/mL and used as the internal standard.

2.3.2. Preparation of calibration curves and quality control samples

The sample concentrations for the calibration curve were 5, 20, 100, 200, and 500 ng/mL, which were prepared by spiking 100 μL of the appropriate standard solution into 900 μL of drug-free whole blood with 30 μL of the internal standard solution (MCPA 0.1 μg/mL). Quality control (QC) samples were prepared by spiking the working standard solutions into drug-free whole blood to evaluate the recovery, matrix effect, process efficiency, precision, accuracy, and stability. In this case, the final concentrations of bentazone and 6- and 8-hydroxybentazone in blood were 20, 200, and 400 ng/mL, respectively.