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Determination of molindone enantiomers in human plasma by high-performance liquid chromatography–tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases

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ABSTRACT

A sensitive and selective bioanalytical assay was developed and validated for the determination of enantiomeric molindone in human plasma using high-performance liquid chromatography–tandem mass spectrometry along with supported liquid extraction procedures. The chiral separation was evaluated and optimized on macrocyclic antibiotic type chiral stationary phases (CSPs) based on teicoplanin aglycone (Chirobiotic TAG) in polar organic, polar ionic, and reversed-phase mode chromatography, respectively. Complete baseline separation was achieved on a Chirobiotic TAG column under isocratic condition in reversed-phase chromatography. The method validation was conducted using a Chirobiotic TAG column (100 mm × 2.1 mm) over the curve range 0.100–100 ng/ml for each molindone enantiomer using 0.0500 ml of plasma sample. The flow rate was 0.8 ml/min and the total run time was 9 min. Supported liquid extraction in a 96-well plate format was used for sample preparation. Parameters including recovery, matrix effect, linearity, sensitivity, specificity, carryover, precision, accuracy, dilution integrity, and stability were evaluated. The intra- and inter-day precision and accuracy of the quality control samples at low, medium, and high concentration levels were RSD ≤3.4% and relative error (RE) ≤4.2% for molindone enantiomer 1, and RSD ≤2.6% and RE ≤5.0% for molindone enantiomer 2, respectively. This validated method could be used for the determination of individual molindone enantiomer in human plasma samples from clinical studies.

1. Introduction

Molindone (Fig. 1), an indole derivative or dihydroindolone, has been used as racemate for the treatment of psychotic symptoms in the USA since1975 [1]. It is structurally different from other classes of antipsychotic agents such as phenothiazines, thioxanthenes, and the butyrophenones [2,3]. However, molindone is as efficacious as other neuroleptics for the treatment of psychotic symptoms due to its ability to block dopamine D_2 receptors, especially for patients with chronic schizophrenia [1,4]. In addition, molindone has been recognized for producing fewer side effects than other antipsychotic agents [5] and for its potential benefit of causing significant weight loss [3,4].

Numerous antipsychotic drugs such as molindone contain one or more chiral centers. The possible contribution of individual enantiomer to a drug's therapeutic and/or adverse effects as well as to drug–drug interactions has gained significant attention in recent years whether the drug was used as racemate or single enantiomer [6–8]. The chirality and drug efficacy of some antipsychotic agents has been reviewed by Lane and Baker [9] and Baker and Prior [10].

The antipsychotic activity of molindone has been reported to be stereospecific, residing in the (−) enantiomer [11]. It is critical, therefore, to know the potential difference in drug metabolism and stereoconversion *in vitro* or *in vivo* for each molindone enantiomer. As a consequence, a reliable chiral assay is necessary to ascertain the potential difference. To date, however, a bioanalytical method for determining the individual molindone enantiomers from biological fluids was not available. The goal of this study was to develop and validate an enantioselective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method based on the use of an appropriate chiral stationary phase for the analysis of biological samples.

Although a tremendous number of LC chiral stationary phases (CSPs) are commercially available, the macrocyclic antibiotics have been demonstrated as some of the most promising chiral selectors since they were originally introduced by Armstrong [12]. These

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antibiotics which include avoparcin, teicoplanin, ristocetin A and vancomycin, and glycopeptides of the vancomycin family, have unique structural features and functionalities that allow various interactions (electrostatic, hydrophobic, H-bonding, steric repulsion, dipole stacking, π–π interaction) between the analyte and the stationary phase. As a result of these diverse mechanisms in enantioseparation, they have been widely used in normal phase, polar organic, polar ionic, and reversed-phase conditions [13–16] for the separation of a wide range of compounds with various polarities [17,18]. To some extent, these macrocyclic glycopeptides are complementary to one another in enantioseparation. This suggests that better separation or baseline separation could be achieved by using different glycopeptides [19,20]. It also has been noted that the aglycone without the attached carbohydrate moieties of teicoplanin and vancomycin were more effective in the separation of certain types of analytes [17,21].

In this study, three macrocyclic glycopeptides CSPs based on native teicoplanin (Chirobiotic T), teicoplanin aglycone (Chirobiotic TAG), and vancomycin (Chirobiotic V) were evaluated in polar organic, polar ionic, and reversed-phase mode chromatography. The conditions that provide the best resolution for enantiomeric separation were evaluated. As a result of this investigation, Chirobiotic TAG was successfully used in a reversed-phase condition for the method validation with the LC run time of 9 min and a complete baseline resolution of enantiomers.

Supported liquid extraction (SLE) in a 96-well plate format was applied for sample preparation. Since this extraction takes approximately 30 min, this assay can be used for high throughput analysis of clinical samples.

2. Experimental

2.1. Chemical, materials, and reagents

Racemic molindone (≥98%) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Deuterium-labeled internal standard (I.S.) of racemic molindone was synthesized in the laboratory as [$^2H_8$]molindone (molindone-d_8) with a purity of 100%. Formic acid (chemical purity ≥96%) was purchased from Sigma–Aldrich. Ammonium formate (certified) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA). HPLC-grade acetonitrile and methyl *tert*-butyl ether (MTBE) were obtained from Sigma–Aldrich. ACS/USP grade ethyl alcohol (ethanol) was obtained from AAPER (Shelbyville, KY, USA). PURELAB Ultra system from ELGA (Marlow, UK) was used in the laboratory to produce deionized water. SLE+ 96-well plates containing 200 mg support media per well, setting limit of the maximum aqueous load of 200 μl, were obtained from Biotage (Uppsala, Sweden). Human plasma with K2-EDTA as the anticoagulant was obtained from Biochemed (Winchester, VA, USA).

2.2. Apparatus

A positive pressure processor (Speedisk 96) used for speeding sample absorption to SLE+ plate was obtained from J.T. Baker. An automated SPE system (Quadra 96 model 96–320) from Tomtec (Hamden, CT, USA) was used to add organic solvents and transfer samples during sample preparation. A 96-well sample concentrator (SPE DRY-96) with temperature control was purchased from Jones Chromatography (Lakewood, CO, USA) for drying samples. The HPLC system consisting of solvent delivery system LC-20AD, autosampler SIL-20AC, column oven CTO-20AC, degasser DGU-20A_3, and controller (CBM-20A) was from Shimadzu (Kyoto, Japan). An API 4000 triple quadrupole mass spectrometer (MDS-Sciex, Concord, Canada) with turboionspray (TIS) interface was used for detection.

2.3. Chromatographic conditions

Chromatographic separation was evaluated using analytical columns (100 mm × 2.1 mm, 5 μm), which were purchased from Supelco (Bellefonte, PA, USA), based on the following CSPs: Chirobiotic teicoplanin aglycone (TAG), Chirobiotic teicoplanin (T), and Chirobiotic vancomycin (V). The separation of molindone enantiomers was further optimized by tuning of the LC mobile phase composition for polar organic mode, polar ionic mode, and reversed-phase mode, respectively. For polar organic mode, methanol, ethanol, and acetonitrile were evaluated. A solution of 10 mM ammonium formate in methanol was used in polar ionic mode. Major efforts were put in optimizing mobile phase conditions (MPCs) to achieve complete baseline separation in reversed-phase mode. Mobile phase A (A) consists of aqueous phase, which can be manipulated to achieve optimum conditions by adjusting its pH value and ionic strength. Mobile phase B (B) was composed of acetonitrile containing 0.02% formic acid. An optimal separation of molindone enantiomers was achieved either by running mobile phase condition 1 (MPC1) consisting of 15% B (0.02% formic acid in acetonitrile) and 85% of A (0.02% formic acid in water) with a pH of 2.9 for 6 min or by running mobile phase condition 2 (MPC2) consisting of 22% of B (0.02% formic acid in acetonitrile) and 78% of A (0.02% formic acid and 10 mM ammonium formate in water) with a pH of 3.8 for 9 min on Chirobiotic TAG column. The sample injection volume was 10 μl. The column temperature and flow rate were optimized to 25 °C and 0.8 ml/min, respectively.

2.4. Mass spectrometric conditions

Positive ionization mode was used for multiple reaction monitoring (MRM) of the LC–MS/MS analyses. The mass spectrometric parameters were optimized to maximize the MRM sensitivity by infusing a 20 ng/ml standard solution of molindone and molindone-d_8, respectively, in acetonitrile/water/formic acid (50/50/0.1, v/v/v) using a Harvard infusion pump (Harvard Apparatus, South Natick, MA, USA). The optimized instrument conditions were as follows: TIS temperature, 550 °C; TIS voltage, 5500 V; curtain gas, 30; nebulizing gas (GS1), 50; TIS gas (GS2), 70; collision gas, 5; declustering potential (DP), 72 V; entrance potential (EP), 10 V; collision energy (CE) 30 eV; collision cell exit potential (CXP) 15 V. The following precursor to product ion transitions were used for the MRM of molindone and molindone-d_8, respectively, at m/z 277.3 → 100.0 and at m/z 285.3 → 108.1 with dwell time of 1200 ms. The mass spectrometer was operated at unit mass resolution for both Q1 and Q3 quadrupoles.

2.5. Preparation of individual molindone enantiomers

To investigate the possible conversion between two enantiomers of molindone in plasma and under sample preparation conditions, neat solution of molindone racemate at 800 ng/ml in acetonitrile/water (1/1, v/v) was applied to prepare individual molindone enantiomers using Chirobiotic TAG column under MPC2 as described in Section 2.3. By post-column splitting, 25% of the flow was switched to and monitored by MS while the remaining portion of flow was split for fractions collection. Fractions of individual molindone enantiomers were collected separately and evaporated to dryness using a 96-well sample concentrator (SPE DRY-96) set at 50 °C. The purified individual molindone enantiomers were reconstituted in acetonitrile/water