Drug Monitoring and Toxicology: A Procedure for the Monitoring of Oxcarbazepine Metabolite by HPLC–UV

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Abstract

This article describes a rapid high-performance liquid chromatographic (HPLC) method for the measurement of the primary metabolite of oxcarbazepine. Following a simple precipitation step, 10,11,-dihydro-10-hydroxy-5H-dibenzo(*b,f*)azepine-5-carboxamide is quantitated (5–60 µg/mL) by analysis on an HPLC–UV system. The instrument time is less than 5 min per injection, an improvement over most published methods. The assay's limit of quantitation, linearity, imprecision, and accuracy adequately cover the therapeutic range for appropriate patient monitoring. In comparison to other published methods, this procedure would be of interest to clinical laboratories because it employs a precipitation step for sample preparation, instead of conventional yet time-consuming solid-phase extraction.

Introduction

Oxcarbazepine (OXC) (Trileptal), is a newer antiseizure drug, released in the United States in 1999. OXC is a keto analogue of carbamazepine. The primary active metabolite of OXC, generated by reduction, is 10,11,-dihydro-10-hydroxy-5H-dibenzo-(*b,f*)azepine-5-carboxamide, also referred to as the monohydroxy metabolite (MHD) (Figure 1). Because of the short half-life of OXC (1 to 2 h), only trace amounts of the parent are detectable in human periphal blood, making MHD the analytical target for monitoring OXC therapy. Although two enantiomers of MHD are formed, pharmacological activity of each enantiomer is similar and thus, separation of the enantiomers for the purpose of therapeutic drug monitoring is not required (1)

Both MHD and OXC produce similar anticonvulsant efficacy, likely because of structural similarities with carbamazepine (2–10). Like carbamazepine, MHD is very effective in the traditional animal seizure models, but less effective in newer animal models, such as pilocarpine- and kainic acid-induced seizures (6). MHD reaches peak plasma levels in 4–6 h and is not extensively metabolized, producing only transdiol carbazepine, a shared metabolite with carbamazepine that is believed to be devoid of anticonvulsant activity. MHD inhibits cytochrome P450 2C19 and induces cytochrome P450 3A4, consequently contributing to several drug–drug interactions (11–15).

More than 95% of OXC is excreted in urine as metabolites, with approximately 27% as MHD and only approximately 1% excreted as unchanged OXC. A linear correlation is seen between the clearance of MHD renally and creatinine clearance. Thus, OXC dosing should be reduced for patients with renal impairment. OXC has been approved for partial and generalized tonic–clonic seizures in adults and children but may have a broader spectrum of use. It is currently under study for management of mood disorders (16,17), post-traumatic stress disorders (18), neuropathic pain (19,20), bipolar disorder (21), and schizophrenia (22).

Several significant adverse effects have been seen with OXC therapy. These include hyponatremia (23,24), the inappropriate secretion of antidiuretic hormone (25), and hypersensitivity (2). Serum sodium concentrations are important to monitor in patients that were administered OXC. MHD is important to monitor clinically when therapy is initiated, with changes to the dosing regimen of OXC or other potentially interacting compounds (or both), especially in patients with compromised renal function. The therapeutic range for MHD is not well established, but a target serum range of 15.0–45.0 mg/L has been proposed. Side effects are reported more frequently in patients with serum concentrations greater than 45 mg/L (26).

A number of methods have been described for measuring OXC and MHD. All methods employ high-performance liquid chromatography (HPLC) with various detection systems and suffer

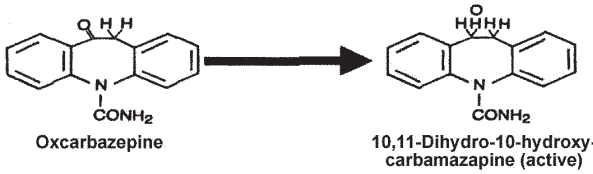

Figure 1. Chemical structures of OXC and MHD.

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from laborious sample set-up procedures utilizing solid-phase extraction (SPE), analytical times of 8 to 30 min, or the use of more expensive mass spectrometric (MS) detectors (27–32). In this paper, a rapid and simple isocratic HPLC assay is described, which uses the commonly available UV detection for the quantitation of MHD with OXC as the internal standard (IS).

Experimental

The assay system included an automated Agilent (Agilent Technologies, Palo Alto, CA) 1100 series LC with autosampler, column heater, and variable UV wavelength detector (240 nm), utilizing Chemstation software (Agilent Technologies) for result recording. Separation was performed on a 250- × 4.6-mm i.d. Synergi Hydro-RP column (Phenomenex, Torrance, CA) with 4-µm spherical particles connected to a C18 4- × 3.0-mm guard column (Phenomenex), maintained at 70°C. The column was made with ultrahigh purity, base-deactivated, type-B silica to allow for minimal metal sites for chelation and reduced silaniol acidity, as well as, to minimize peak tailing of basic compounds. The 80-Å pore size increases the surface area, allowing adequate interaction for highly polar analytes and compensating for its reduced hydrophobicity, allowing it to retain both hydrophobic and hydrophilic analytes through polar and nonpolar interactions. The column is designed to be stable in aqueous mobile phases, allowing for more stringent separation (33). The mobile phase (isocratic, 1.1-mL/min flow rate) consisted of 55% 0.01M potassium phosphate pH 7.0, 20% methanol, and 30% acetonitrile.

HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Hampton, NH). Potassium phosphate was purchased from Sigma-Aldrich (Dallas, TX). The mobile phase was filtered and degassed through a 0.45-µm nylon membrane under vacuum. The IS, OXC, and MHD were gifts from Novartis Pharmaceuticals Corporation (Basel, Switerland). The primary stock solutions (100 g/L) of MHD and OXC were prepared in methanol.

Samples were prepared by transferring 0.15 mL of patient serum or plasma, positive control, negative control (blank), and calibrators into respective microcentrifuge tubes. Then 50 µL of the 20 ng/µL OXC solution was added to each tube followed by 0.20 mL of methanol. The tubes were capped and vortexed for approximately 30 s, then centrifuged for 10 min at approximately 2500 × *g*. The supernatant was transferred to an autosampler vial, capped tightly, and 40 µL was then injected onto the column. A ratio of the peak height of the analyte divided by the peak height of OXC was used to quantitate the analytes of interest from the calibration curve.

Results and Discussion

The validation of this method involved consideration of the linearity and analytical measurement range, accuracy, precision, potential for interferences, and carryover. Validation samples included spikes prepared with C18 filtered blood bank plasma and clinically relevant concentrations of MHD, as well as residual serum specimens previously analyzed for MHD by a nationally recognized reference laboratory or by our laboratories for other drugs.

The linearity of the method was evaluated by analyzing C18 filtered blood bank plasma to which MHD was added at the following concentrations: 1, 10, 20, 40, 80, and 100 mg/L. This experiment was performed three times over a period of 2months. All calibrators were sent to a reference laboratory to validate concentrations. Each linearity sample was then analyzed in duplicate and concentrations were calculated from a five-point calibration curve (1, 10, 20, 40, and 60 mg/L). Accuracy between 1 and 100 mg/L, the highest concentration evaluated, was between 85–115% of the spiked value. The total coefficient of variation (CV) averaged 2.8%, and a least squares linear regression equation:

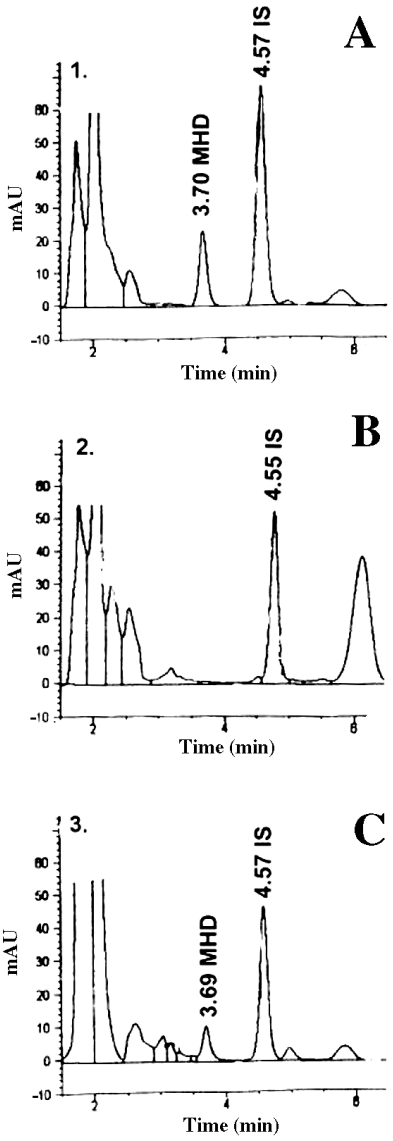

Figure 2. Chromatograms obtained from the 10 mg/L calibrator (A), a blank (B), and a representative patient on OXC therapy (C) are shown. The peaks of interest are MHD at 3.7 min and the IS (OXC) at 4.5 min for a total instrument run time of 5.0 min.

$$y = 0.998x - 0.08 \quad \text{Eq. 1}$$

where $r = 0.998$ with a standard error value of 1.37.

At 1 mg/L MHD, accuracy was between 84% and 109% and the total CV was 4.5% ($n = 36$). This concentration was determined as the lowest concentration tested that produced a peak three times as great as the baseline noise of a plasma blank. Figure 2 illustrates chromatograms obtained from the 10 mg/L calibrator (Figure 2A), a blank (Figure 2B), and a representative patient on OXC therapy (Figure 2C). The peaks of interest are MHD at 3.70 min and OXC at 4.5 min for a total instrument run time of 5.0 min. Carryover was not observed after injecting a sample (100 mg/L) followed by blank plasma over three separate runs, over 3 days.

To check for interferences, 25 randomly selected de-identified patient samples were analyzed. No interferences were found with therapeutic concentrations of carbamazepine, both the epoxide and hydroxy metabolites, zonisamide; levetircaetam, gabapentin, vigabitrin, phenytoin and metabolites, felbamate, lamotrigine, clonazepam, phenobarbital, primidone, acetaminophen, salicylate, ibuprofen, amitriptyline, nortriptyline, desipramine, doxepin and nordoxepin, imipramine, valproic acid, topiramate, mephenytoin, amiodarone and desethylamiodarone, methsuximide and normethsuximide, ethotoin, clozapine, diazepam and nordiazepam, lorazepam, nitrazepam, and sertraline. Amlodipine and metformin have the potential, in overdose concentrations, of smearing across the MHD peak, but they were separated well at therapeutic concentrations.

Forty residual samples submitted for OXC testing with MHD concentrations between 5 and 50 ng/mL were injected without adding OXC to detect interfering levels of OXC. None of the samples were found to contain measurable amounts of OXC, thus supporting the use of OXC as the IS. Nonetheless, peak heights must be monitored within each run to identify an interference that might appear in acute OXC overdose or other situations wherein OXC may not have converted to MHD prior to specimen collection.

To further validate the accuracy of this method, 30 de-identified patient samples with MHD concentrations ranging from 5 to 60 mg/L were split, and the results were compared with a nationally recognized reference laboratory that employed HPLC–MS. The least squares linear regression equation for correlation where y is the HPLC method described here was:

$$y = 1.05x + 0.29 \quad \text{Eq. 2}$$

where $r = 0.97$ with a standard error value of 2.10. These data are shown in Figure 3.

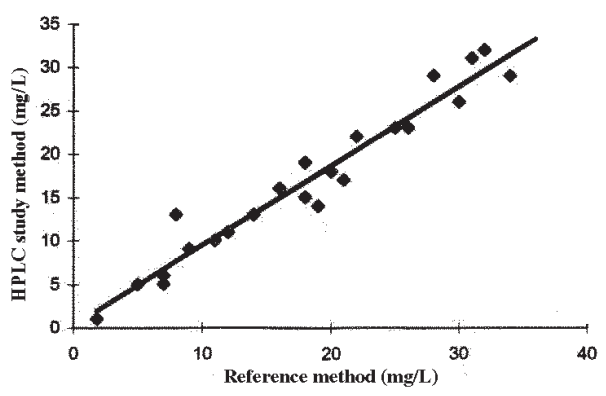

Figure 3. Method comparison of results obtained with 30 de-identified patient samples are plotted with regression statistics. The MHD concentrations (mg/L) are shown for each sample analyzed in both methods.

To avoid the use of SPE and the time associated with it, a methanol precipitation method was chosen. Methanol was chosen over acetonitrile to improve extraction efficiency. Specifically, pellet formation with acetonitrile was observed prior to vortex mixing, a phenomenon that reduced extraction efficiency (data not shown). The Hydro-RP column at 70°C was stable for over 1000 injections (data not shown) and showed significantly lower back pressure than a standard C18 column. The recovery was comparable to the 80–100% recovery commonly published with SPE methods (33).

Conclusion

This report describes a robust assay for the measurement of MHD by HPLC–UV. The Hydro-RP column shows increased hydrophobic and polar retention when compared with standard C18 columns and performed well under a higher aqueous mobile phase. Column lifetime generally supercedes 1000 injections, with the precolumn being replaced approximately every 150 injections. The extraction process avoids the use of SPE, which substantially decreases set-up time in comparison with other published methods. It has been proven to be a reliable chromatographic assay alternative to current methods by performing as well as more expensive MS detectors and could likely be successfully placed into a small clinical laboratory.

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