Drug Monitoring: Simultaneous Analysis of Lamotrigine, Oxcarbazepine, 10-Hydroxycarbazepine, and Zonisamide by HPLC–UV and a Rapid GC Method Using a Nitrogen-Phosphorus Detector for Levetiracetam

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Abstract

A high-performance liquid chromatography (HPLC) assay using UV detection is described for the simultaneous measurement of the newer generation anti-epileptic medications lamotrigine, oxcarbazepine (parent drug and active metabolite 10-hydroxycarbazepine), and zonisamide. Detection of all four compounds can be done at 230 nm; however, there is a potential interference with zonisamide in patients on clonazepam therapy. Therefore, the method uses dual wavelength detection: 230 nm for oxcarbazepine and 10-hydroxycarbazepine and 270 nm for lamotrigine and zonisamide. In addition, a simple gas chromatography method using a nitrogen-phosphorus detector is described for the measurement of levetiracetam, another of the recently approved anti-epileptic medications. For both methods, limits of quantitation, linearities, accuracies, and imprecisions cover the therapeutic range for drug monitoring of patients. A wide variety of clinical drugs, including other anti-epileptic drugs, do not interfere with these assays. These procedures would be of special interest to clinical laboratories, particularly due to the limited availability of immunoassays for newer generation anti-epileptic medications and that therapeutic uses of these drugs are expanding beyond epilepsy to other neurologic and psychiatric disorders.

Introduction

During the past twelve years, a number of novel anti-epileptic agents have been introduced into clinical practice in the United States, considerably expanding the therapeutic options beyond traditional medications such as carbamazepine, phenobarbital, phenytoin, primidone, and valproic acid. For therapeutic drug monitoring, immunoassays for the traditional anti-epileptic drugs have been available for years from multiple companies, in many cases on high-throughput chemistry analyzers. In contrast, development of similar assays for the newer anti-epileptic agents has been slow. Unlike the traditional anti-convulsants, immunoassays for the newer anti-convulsants, if commercially available, tend to run on a limited number of specialized platforms and not on common high throughput chemistry analyzers. This necessitates the development of robust and straightforward chromatographic assays for the new anti-epileptic agents. This article reports methods appropriate for drug monitoring of four of the most frequently prescribed of the newer anti-epileptic medications: lamotrigine, levetiracetam, oxcarbazepine, and zonisamide. The chemical structures of these four drugs, along with the two major metabolites of oxcarbazepine, are shown in Figure 1.

Lamotrigine (Lamictal, GlaxoSmithKline) is a broad-spectrum anti-epileptic drug of the phenyltriazine class chemically unrelated to other anti-convulsants that was approved by the Food and Drug Administration (FDA) for treatment of epilepsy in 1994 (1,2). In 2003, lamotrigine received an additional approval for treatment of bipolar disorder. Lamotrigine is predominantly
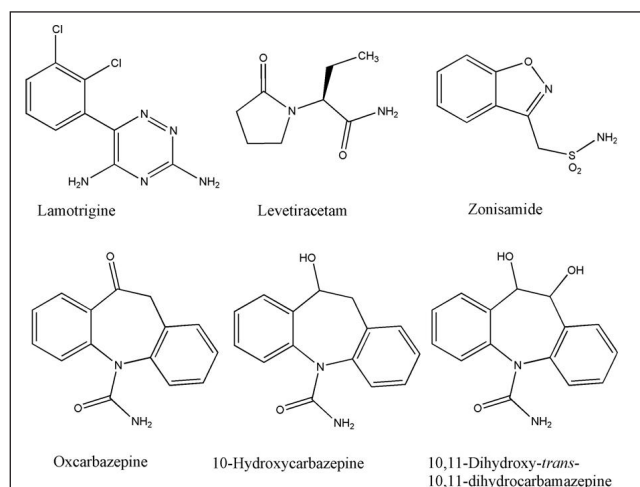

Figure 1. Chemical structures of lamotrigine, levetiracetam, zonisamide, oxcarbazepine, 10-hydroxycarbazepine (pharmacologically active metabolite of oxcarbazepine), and 10,11-dihydroxy-*trans*-10,11-dihydrocarbamazepine (therapeutically inactive metabolite of oxcarbazepine).

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metabolized in the liver by glucuronidation (3). Lamotrigine has an average elimination half-life of 33 h, although this can be influenced by concomitant therapy with other medications that inhibit or induce expression of hepatic drug-metabolizing enzymes. Therapeutic drug monitoring of lamotrigine targets the parent drug as little clinical benefit has been found for monitoring the glucuronide metabolite (3). Several methods have been used for determination of lamotrigine concentrations including high-performance liquid chromatography (HPLC) (4–8), gas chromatography (GC) with a nitrogen-phosphorus detector (NPD) (9), and radioimmunoassay (10).

Levetiracetam (Keppra, UCB Pharma) is an anti-epileptic drug licensed for the treatment of partial seizures in adults and is sometimes used in combination with other anti-epileptic drugs. It is also effective in children with partial seizures and may also be useful in select patients with generalized seizures (11). The mechanism of action of levetiracetam is unclear but may involve inhibition of voltage-activated calcium channels in the brain. The elimination half-life of levetiracetam is approximately 6 to 8 h in adults. Approximately 66% of the drug is excreted unchanged and 27% as an inactive hydrolysis product. Levetiracetam is predominantly cleared by the kidneys so that elimination parallels kidney function (3,12). Levetiracetam is now available in an intravenous formulation in both Europe and the United States, allowing this drug to be used more frequently in urgent clinical situations such as status epilepticus, where faster turnaround time on levetiracetam plasma concentrations may be needed clinically. A number of methods have been developed for determination of levetiracetam concentrations including HPLC (13–17), gas chromatography (18) (note that little experimental detail was provided in this report as analysis was done by a contract research organization), GC–mass spectrometry (MS) (19), and liquid chromatography–MS–MS (20). Levetiracetam raises some challenges with extraction and HPLC analysis due to the compound's high polarity and lack of a good chromophore for UV absorption (14,15). HPLC analysis of levetiracetam, thus, requires either derivatization or use of shorter wavelength UV light that other compounds absorb non-specificity.

Oxcarbazepine (Trileptal, Novartis) is the 10-keto analog of carbamazepine with similar therapeutic effects but with fewer adverse effects, including less ability to induce the expression of hepatic drug-metabolizing enzymes (1,2). Oxcarbazepine is now a first-line drug for the management of partial seizures. Like carbamazepine, oxcarbazepine blocks voltage-gated sodium channels. Following oral administration and absorption, oxcarbazepine is rapidly reduced by cytosolic arylketone reductases to its active metabolite 10-hydroxy-10,11-dihydrocarbamazepine (10-hydroxycarbazepine). 10-Hydroxycarbazepine accounts for the majority of anti-epileptic activity following oxcarbazepine administration, and, therefore, therapeutic drug monitoring generally targets this metabolite (3,21). 10-Hydroxycarbazepine is eliminated by conjugation with glucuronic acid. Minor amounts (4% of the ingested dose of oxcarbazepine) are further oxidized from 10-hydroxycarbazepine to 10,11-dihydroxy-*trans*-10,11-dihydrocarbamazepine, which is pharmacologically inactive with respect to treating seizures (21). A number of methods have been used to determine oxcar-

bazepine and 10-hydroxycarbazepine metabolite concentrations including HPLC (8,22–28), GC (29), and micellar electrokinetic chromatography (30).

Zonisamide (Zonegran, Eisai Co.) is an anti-epileptic drug licensed in the United States for the treatment of partial seizures in adults (1,2). Zonisamide is also used in Lennox-Gestaut syndrome (often a treatment-refractory type of epilepsy), West's syndrome (a form of infantile epilepsy), bipolar disorder, and migraine headaches. Zonisamide has an elimination half-life of approximately 50–70 h in adults and is extensively metabolized by acetylation and conjugation. Zonisamide is a substrate of cytochrome P450 2C19 and 3A4 and is subject to drug–drug interactions with inhibitors or inducers of these liver enzymes (3). Therapeutic drug monitoring of zonisamide has involved determination of serum/plasma concentrations of only the parent compound, as no advantage has been found in monitoring metabolite concentrations (3). Several HPLC methods have been reported for zonisamide (14,31,32).

An HPLC method that simultaneously measures lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide concentrations in plasma/serum has not been reported. These medications are increasingly being used in settings outside of epilepsy such as bipolar disorder and migraine headaches and sometimes in combination for refractory epilepsy or other conditions where serum/plasma levels of multiple drugs are needed simultaneously. Therefore, having an assay that can analyze multiple drugs simultaneously with a single extraction is very helpful in increasing throughput and limiting manual steps. In this paper, an HPLC–UV assay is described that accomplishes this goal. In addition, a GC assay using a NPD for levetiracetam is described that can support rapid turnaround of levetiracetam plasma concentrations. This assay uses a methylene chloride-based extraction that achieves excellent specificity.

Experimental

Reagents

Lamotrigine was generously supplied by GlaxoSmithKline (Triangle Park, NC). 10-Hydroxycarbazepine and 10,11-dihydroxy-*trans*-10,11-dihydrocarbamazepine were kindly supplied by Novartis (Basel, Switzerland). Levetiracetam reference standard was generously supplied by UCB Pharma (Smyrna, GA). Oxcarbazepine, zonisamide, and chloramphenicol were obtained from Sigma-Aldrich (St. Louis, MO). Mepivacaine (1 mg/mL in methanol) was purchased from Alltech (Lancaster, PA). Acetonitrile, methanol, and water were HPLC grade (Fisher Scientific, Pittsburgh, PA). Ethylacetate, potassium phosphate, methylene chloride, and phosphoric acid were analytical grade (Fisher). Drug-free human plasma was obtained from the University of Pittsburgh Medical Center Central Blood Bank.

HPLC analysis of lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide

The HPLC system consisted of a Waters 717 Plus Autosampler and Systems Controller and a Waters 2487 Dual Absorbance Detector (Milford, MA). The separation was performed at 22°C

with a Supelcosil LC-18 column (25 cm × 4.6 mm internal diameter, 5 μM silica particles; Supelco, Bellefonte, PA). The mobile phase was a mixture of aqueous 3mM potassium phosphate buffer (adjusted to pH 3.7 with 5% phosphoric acid) and acetonitrile (65:35) at a flow rate of 1.2 mL/min. Oxcarbazepine and 10-hydroxycarbazepine are monitored at 230 nm while lamotrigine and zonisamide are monitored at 270 nm (all other HPLC conditions are identical for the four compounds). Stock solutions of oxcarbazepine, 10-hydroxycarbazepine, lamotrigine, zonisamide, and the internal standard chloramphenicol were at 1000 μg/mL in HPLC grade methanol. To 250 μL of sample (calibrator, control, or patient sample) in a 16 × 125 mm glass culture tube, 100 μL of chloramphenicol solution, 1.5 mL 10 mM NaOH, and 4.0 mL ethylacetate were added. The samples were immediately vortexed for 1 min and centrifuged at $1,700 \times g$ for 5 min. The organic (upper) layer was transferred to a conical tube and dried completely at 40°C using a nitrogen evaporator. The sample was then reconstituted with 100 μL mobile phase, vortexed, and transferred to an autosampler vial for HPLC analysis. A range of possible interfering substances were evaluated by extracting and analyzing Liquichek Immunoassay Plus Control (Bio-Rad, Irvine, CA). This serum product has over 90 compounds, including therapeutic drugs (e.g., acetaminophen, amikacin, amiodarone, amitriptyline, caffeine, carbamazepine, cyclosporine, desipramine, digoxin, disopyramide, flecainide, gentamicin, ibuprofen, imipramine, lidocaine, lithium, netilmycin, nortriptyline, propanolol, quinidine, salicylate, tobramycin, and valproic acid), vitamins, and steroid hormones. Other compounds not found in the Liquichek product were also screened for interference, particularly neurologic and psychiatric medications such as baclofen, clonazepam, felbamate, tiagabine, and topiramate.

GC analysis of levetiracetam

The GC system was a Perkin-Elmer Autosystem XL GC with NPD and a HP-5MS capillary column (5% phenyl methyl siloxane, 30 m × 0.251 mm × 0.25 μM; J&W Scientific, Folsom, CA). The internal standard mepivacaine solution was prepared at 10 μg/mL in methanol. Several different possible extraction procedures were evaluated before determining that extraction with methylene chloride provided excellent specificity and recovery. Extraction with ethylacetate would be an alternative but leads to less specificity. To 250 μL of sample (calibrator, control, or patient samples) in a 16 × 125 mm screw-top culture tube, 50 μL of mepivacaine solution, 250 μL of phosphate buffer (290 mM KH_2PO_4, 210 mM Na_2HPO_4, adjusted to pH 6.4 with 10% phosphoric acid), and 5 mL methylene chloride were added. The samples were immediately vortexed for 1 min and centrifuged at 2,400 rpm for 5 min. The bottom organic layer was then transferred to a 10 mL conical centrifuge tube and evaporated to dryness at 40°C using a nitrogen evaporator. The sample was then reconstituted with 50 μL methanol, vortexed, and transferred to an autosampler vial for GC analysis. The oven temperature began at 90°C, increased by 42.0°C/min to 190°C, and then increased by 10.0°C/min to 250°C. Total analysis time was 8 min. Different temperatures and ramping speeds were extensively evaluated. Attempts to shorten the GC run-time by increasing the ramp speed degraded the quality of the chromatograms. Levetiracetam

is detected poorly by a flame ionization detector. Our initial attempts to use a flame ionization detector showed sensitivity that was at least ten-fold less than that with a NPD. The limit of quantitation using a flame ionization detector is estimated at approximately 10 μg/mL, insufficiently low to capture the therapeutically meaningful range of serum/plasma concentrations. Interfering substances were assessed as described for the HPLC method described.

Determination of recovery, intra-day and inter-day precision, accuracy, linearity, and limit of detection and quantitation

Intraday precision and accuracy were evaluated by the analysis of spiked samples. The precision and accuracy for interday comparisons were assessed at the same concentration and summarized as coefficient of variation (CV%) and relative deviation (RD%), respectively. Linearity of the methods were assessed by analyzing drug-free human plasma to which analytes were added. Calibrators were sent to a reference laboratory to validate concentrations. The linear range was considered to be the concentration range for which the calculated concentrations were within 85–115% of the spiked concentration. Limit of detection was determined using the signal-to-noise of 3:1 by comparing test results from samples with known concentrations of analytes with drug-free plasma (blank) samples. The limit of quantitation was defined as the lowest drug concentration that can be determined reproducibly (coefficient of variation < 20%) and accurately (percent error < 20%).

Results and Discussion

HPLC assays for lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide

Lamotrigine absorbs UV light well in the 270–306 nm range, and a number of HPLC methods utilize wavelengths within this range for detection of lamotrigine (7,33). Fewer methods describe HPLC detection of zonisamide, but this compounds optimally absorbs UV light at a somewhat lower UV range (32,34). Oxcarbazepine and 10-hydroxycarbazepine absorb poorly at 270 nm but better absorption occurs at 215–240 nm (22,25,27).

Under the described extraction and HPLC run conditions, lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide could all be adequately detected at 230 nm (Figure 2A). However, during method development, substantial interference with the zonisamide peak was noted at 230 nm wavelength detection in several patients who were also receiving clonazepam therapy. Testing of blank plasma spiked with clonazepam showed that the parent drug did not account for the interference. We speculate that clonazepam metabolites likely contributed to the interference in these patients, but there is a lack of standards of these metabolites to further test this hypothesis.

Therefore, we searched for two wavelengths that could be used for detection, while still keeping the same extraction and HPLC conditions. A solution was found with 230 nm for oxcarbazepine and 10-hydroxycarbazepine and 270 nm for lamotrigine and zon-

isamide. Using this method, the retention times of 10-hydroxy-carbazepine, zonisamide, chloramphenicol (internal standard), oxcarbazepine, and lamotrigine were 3.5, 4.5, 4.9, 5.2, and 5.9 min, respectively (Figures 2A and 2B), using drug-free plasma spiked with pure compounds. An example of a chromatogram from the plasma of a patient chronically taking both lamotrigine and zonisamide is shown in Figure 3A (using 270 nm wavelength detection). An example of a chromatogram from the plasma of a patient chronically taking oxcarbazepine is shown in Figure 3B (using 230 nm wavelength detection); in this example, most of the parent oxcarbazepine had been converted to 10-hydroxycarbazepine in this patient, as is typical in samples received for therapeutic drug monitoring. The use of a dual-wavelength detector allows for rapid simultaneous analysis of 10-hydroxycarbazepine, zonisamide, chloramphenicol (internal standard), oxcarbazepine, and lamotrigine. Alternatively, for laboratories that have a single variable wavelength detector, the single extraction can still be used with separate injections and analyses at 230 nm (10-hydroxycarbazepine and oxcarbazepine) and 270 nm (lamotrigine and zonisamide).

A wide variety of therapeutic drugs were tested for interference at both 230 and 270 nm, including other anti-epileptic and neurologic medications (and in some cases their metabolites) such as baclofen, carbamazepine, clonazepam (no interference at 270 nm, see previously for description of interference at 230 nm), epoxycarbamazepine, ethosuximide, felbamate, gabapentin, levetiracetam, phenobarbital, phenytoin, primidone, tiagabine, topiramate, and valproic acid. None of the therapeutic drugs tested exhibited any interference. In addition, no peak interferences were found in any of the batches of drug-free plasma. Separate experiments with the reference standard of the dihydroxy metabolite of oxcarbazepine (10,11-dihydroxy-*trans*-10,11-dihydrocarbamazepine) showed that this oxcarbazepine metabolite did not interfere with the HPLC analysis. As described in the introduction, this metabolite is pharmacologically inactive with respect to epilepsy therapy, rarely detected in patients
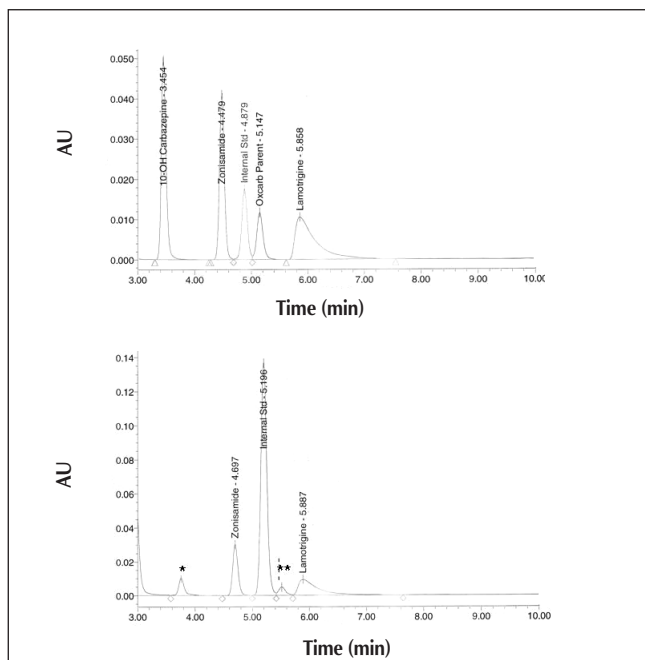

Figure 2. Chromatogram from HPLC analysis of a blank plasma sample spiked with lamotrigine (5 µg/mL), zonisamide (15 µg/mL), oxcarbazepine (2 µg/mL), 10-hydroxycarbazepine (15 µg/mL) and the internal standard chloramphenicol using 230 nm wavelength detection. Note that all compounds are readily detected at this wavelength but potential interference with zonisamide occurs in patients taking clonazepam (not shown) (A). Chromatogram from HPLC analysis of a blank plasma sample spiked with lamotrigine (5 µg/mL), zonisamide (15 µg/mL), oxcarbazepine (2 µg/mL), 10-hydroxycarbazepine (15 µg/mL) and the internal standard chloramphenicol using 270 nm wavelength detection (B). At this wavelength, 10-hydroxycarbazepine (*) and oxcarbazepine (**) are poorly detected compared to 230 nm.
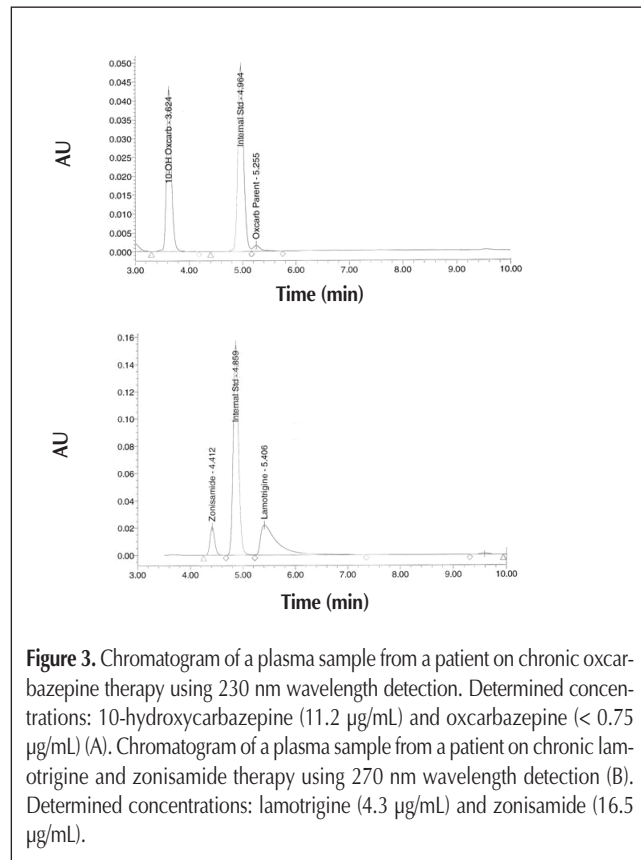

Figure 3. Chromatogram of a plasma sample from a patient on chronic oxcarbazepine therapy using 230 nm wavelength detection. Determined concentrations: 10-hydroxycarbazepine (11.2 µg/mL) and oxcarbazepine (< 0.75 µg/mL) (A). Chromatogram of a plasma sample from a patient on chronic lamotrigine and zonisamide therapy using 270 nm wavelength detection (B). Determined concentrations: lamotrigine (4.3 µg/mL) and zonisamide (16.5 µg/mL).
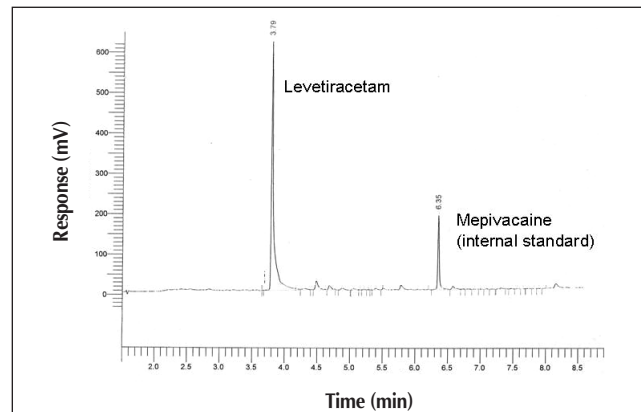

Figure 4. Chromatogram from GC analysis of a plasma sample from a patient on chronic levetiracetam therapy. Determined levetiracetam concentration: 18.8 µg/mL.

on oxcarbazepine therapy, and not of interest for therapeutic drug monitoring purposes.

The method exhibited a good linearity over a concentration range of 1–30 µg/mL for lamotrigine, 1–30 µg/mL for oxcarbazepine, 1–40 µg/mL for 10-hydroxycarbazepine, and 1–40 µg/mL for zonisamide. This covers the therapeutic range for these compounds. For lamotrigine, the linear regression equation for comparison of determined versus spiked concentration was: $y = 1.0x + 0.40$, with $r = 0.998$ and a standard error value of 0.62. For 10-hydroxycarbazepine, the linear regression equation for comparison of determined versus spiked concentration was: $y = 0.997x – 0.07$, with $r = 0.997$ and a standard error value of 0.99. For oxcarbazepine, the linear regression equation for comparison of determined versus spiked concentration was: $y = 0.998x + 0.14$ with $r = 0.996$ and a standard error value of 1.23. For zonisamide, the linear regression equation for comparison of determined versus spiked concentration was: $y = 0.998x + 0.19$, with $r = 0.998$ and a standard error value of 1.36.

Results of intra- and interday accuracy and precision are shown in Table I. The lower limit of detection was 0.15 µg/mL for lamotrigine, 0.25 µg/mL for oxcarbazepine, 0.15 µg/mL for 10-hydroxycarbazepine, and 0.75 µg/mL for zonisamide. The limit of quantitation was 0.5 µg/mL for lamotrigine, 0.75 µg/mL for oxcarbazepine, 0.5 µg/mL for 10-hydroxycarbazepine, and 0.5 µg/mL for zonisamide.

Thirty-eight de-identified patient samples testing positive for lamotrigine were sent to a nationally recognized reference laboratory for comparison studies. The linear regression equation for correlation, with y as the HPLC method described here is $y = 0.98x + 0.12$, with $r = 0.99$ and a standard error value of 0.61. Twenty de-identified patient samples testing positive for 10-hydroxycarbazepine were sent to a nationally recognized reference laboratory for comparison studies. The linear regression equation for correlation, with y as the HPLC method described here is $y = 0.97x + 1.22$, with $r = 0.98$ and a standard error value of 1.44. Twenty de-identified patient samples testing positive for zonisamide were sent to a nationally recognized reference laboratory for comparison studies. The linear regression equation for correlation, with y as the HPLC method described here is $y = 1.0x + 0.86$, with $r = 0.99$ and a standard error value of 1.88.

GC assay for levetiracetam

Under the described GC conditions, the retention times of levetiracetam and mepivacaine (internal standard) were 3.8 and 6.4 min, respectively (Figure 4, patient sample). Evaluation of a number of different extraction methods demonstrated that a simple extraction with methylene chloride provided excellent recovery and specificity for levetiracetam. Other studies that have used fast precipitation methods suffer from lost of specificity and lower sensitivity (15). The methylene chloride extraction presented here is

Table I. Intra- and Interday Accuracy and Precision for the HPLC Assay for Detetermination of Lamotrigine, Zonisamide, Oxcarbazepine, and 10-Hydroxycarbazepine Concentrations in Human Plasma/Serum (All Concentrations in g/mL)

Lamotrigine			
Intraday ($n = 5$)			
Nominal concentration	5	12	20
Mean	5.22 ± 0.23*	12.32 ± 0.61	12.32 ± 0.61
Accuracy as RD (%)	4.4	2.6	1.3
Precision as CV (%)	4.4	4.9	5.5
Interday ($n = 12$)			
Nominal concentration	7	12	
Mean	7.28 ± 0.35	12.10 ± 0.76	
Accuracy as RD (%)	4.0	0.8	
Precision as CV (%)	4.8	6.3	
Zonisamide			
Intraday ($n = 5$)			
Nominal concentration	5	20	30
Mean	4.86 ± 0.37	20.24 ± 0.14	31.0 ± 2.5
Accuracy as RD (%)	2.9	1.2	3.2
Precision as CV (%)	7.6	0.7	8.2
Interday ($n = 12$)			
Nominal concentration		20	30
Mean		20.69 ± 1.6	31.15 ± 2.4
Accuracy as RD (%)		3.3	3.8
Precision as CV (%)		7.9	7.8
Oxcarbazepine			
Intraday ($n = 5$)			
Nominal concentration	10	20	30
Mean	9.93 ± 0.58	20.46 ± 1.28	30.72 ± 1.70
Accuracy as RD (%)	0.7	1.3	2.3
Precision as CV (%)	5.9	6.3	5.5
Interday ($n = 10$)			
Nominal concentration	10	20	
Mean	10.26 ± 0.76	20.19 ± 0.99	
Accuracy as RD (%)	2.6	1.0	
Precision as CV (%)	7.4	4.9	
10-Hydroxycarbazepine			
Intraday ($n = 5$)			
Nominal concentration	5	15	30
Mean	4.70 ± 0.41	14.83 ± 0.43	31.12 ± 1.69
Accuracy as RD (%)	6.4	1.1	3.6
Precision as CV (%)	8.9	2.9	5.5
Interday ($n = 10$)			
Nominal concentration		15	30
Mean		15.18 ± 0.76	29.92 ± 1.49
Accuracy as RD (%)		1.2	0.3
Precision as CV (%)		5.1	5.0

* Values are reported as mean ± SD.

Table II. Intra- and Interday Accuracy and Precision For the GC Assay for Detetermination of Levetiracetam Concentrations in Human Plasma/Serum (All Concentrations in μg/mL)

Levetiracetam			
Intraday (n = 5)			
Nominal concentration	5	12.5	25
Mean	4.74 + 0.28*	12.37 ± 0.73	23.52 ± 1.47
Accuracy as RD (%)	5.5	1.0	6.3
Precision as CV (%)	5.9	5.9	6.3
Interday (n = 10)			
Nominal concentration		12.5	25
Mean		12.25 ± 0.92	25.47 ± 1.66
Accuracy as RD (%)		2.0	1.9
Precision as CV (%)		7.5	6.5

* Values are reported as mean ± SD.

equivalent in amount of effort to published solid-phase extraction methods (14,15) but likely cheaper in cost. The run-times are faster or similar to other published methods for levetiracetam (14–17,19). A wide variety of therapeutic drugs were tested for interference, including other anti-epileptic and neurologic medications (and in some cases their metabolites) such as baclofen, carbamazepine, clonazepam, epoxycarbamazepine, ethosuximide, felbamate, gabapentin, 10-hydroxycarbazepine, 10,11-dihydroxy-*trans*-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, primidone, tiagabine, topiramate, valproic acid, and zonisamide. None of the therapeutic drugs tested exhibited any interference. In addition, no peak interferences were found in any of the batches of drug-free plasma. Twenty de-identified patient samples testing positive for levetiracetam were sent to a nationally recognized reference laboratory for comparison studies. The linear regression equation for correlation, with y as the HPLC method described here is $y = 0.87x + 1.47$, with $r = 0.96$ and a standard error value of 1.59.

The method exhibited a good linearity over a concentration range of 2.5–45 μg/mL, covering the therapeutic range. The linear regression equation for comparison of determined versus spiked concentration was: $y = 0.99x + 0.35$, with $r = 0.998$ and a standard error value of 1.11. Results of intra- and interday accuracy and precision are shown in Table II. The lower limit of detection was 0.5 μg/mL and the limit of quantitation was 1.5 μg/mL. The limits of detection and quantitation are similar to or lower than previously reported HPLC (14,16) or GC assays (18).

Conclusions

The HPLC method described here is simple, sensitive, and specific and allows for the simultaneous determination of three commonly prescribed newer anti-epileptic drugs (including the pharmacologically active metabolite of oxcarbazepine). The GC method using a nitrogen-phosphorus detector is sensitive and specific for levetiracetam and is suitable for clinical laboratory use.

Acknowledgments

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