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Development and validation of a GC/MS method for the simultaneous determination of levetiracetam and lamotrigine in whole blood

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ABSTRACT

A sensitive and accurate gas chromatography–mass spectrometric method was developed and validated for the simultaneous determination of levetiracetam and lamotrigine in whole blood. A solid-phase extraction (SPE) procedure using HF Bond Elut C18 columns followed by derivatization using N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylsilyl chloride (TBDMSCl) was used. In this assay, levetiracetam-d6 was used as internal standard. Limits of detection and quantification were 0.15 and 0.50 μg/mL, respectively, for both analytes. The method was proved to be linear within the concentration range of 0.50–50.0 μg/mL ($R^2 \geq 0.992$) for both analytes. Absolute recovery was found to be at least 90.0 and 97.2% for levetiracetam and lamotrigine, respectively. Intra-day and inter-day accuracy values for both analytes were ranged from −6.5 to 4.2 and −6.6 to 3.0%, respectively, whereas their respective precision values were less than 11.4 and 8.3%. The developed method was successfully used in our laboratory for quantification of levetiracetam and lamotrigine blood concentrations during the investigation of forensic cases where these antiepileptic drugs were involved. This method could also be used for therapeutic drug monitoring purposes.

1. Introduction

Epilepsy is a common chronic neurological disorder that is characterized by recurrent, unprovoked seizures, due to excessive electrical activity in the brain [1,2]. In epilepsy treatment, anticonvulsant drugs are normally used to prevent seizures. For patients who have insufficient seizure control with their conventional treatment, an adjunctive antiepileptic drug is also needed [3]. Therapeutic drug monitoring (TDM) of antiepileptic drugs is useful in various settings, such as when drug interactions are expected, toxicity is suspected, or when newer antiepileptics with non-clear pharmacokinetics are used. Dosage regimens of antiepileptics should be assessed regularly, and adjusted if necessary, so that patients can derive optimal therapeutic benefits [4].

Levetiracetam [(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a relatively new antiepileptic drug that is chemically unrelated to the traditional antiepileptic drugs in current use [3,5]. It is considered to have several advantages over other antiepileptics, as it is highly effective, and it is indicated as monotherapy or as an adjunctive therapy in the treatment of partial-onset seizures of chronic epilepsy. It is also useful, alone or in combination with other drugs, for the treatment of bipolar disorders, mania and migraine [4]. Levetiracetam may be particularly useful in patients who are not responding to other antiepileptics, patients receiving multi-drug treatment with a high potential for developing drug interactions, or those with hepatic impairment [6]. The efficacy is dose-dependent and usually the daily therapeutic dosage of levetiracetam ranges from 1000 to 3000 mg [7], with a respective serum concentration range of about 10–50 μg/mL [3,4]. Although, levetiracetam seems to be well tolerated, adverse effects are still possible during treatment. Due to its recent introduction in therapy, its pharmacological–toxicological profile has not been fully studied yet so, TDM of patients undergoing levetiracetam therapy is advisable, especially in children and the elderly. Levetiracetam exhibits rapid absorption following oral administration, good bioavailability, quick attainment of steady-state concentrations, linear pharmacokinetics, minimal plasma protein-binding, insignificant hepatic metabolism giving inactive, renally excretable

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metabolites and consequently lack of interactions with other drugs [2,3,5]. The mechanism of action of levetiracetam is unclear but may involve inhibition of voltage-activated calcium channels in the brain [8]. Unlike other antiepileptic drugs, the metabolism of levetiracetam does not include the cytochrome P450 system. Plasma half-life of levetiracetam is relatively short (approximately 6–8 h) and it is primarily excreted in urine [2,9]. Sixty-six percent (66%) of the administered dose is excreted unchanged renally, while approximately 24% of levetiracetam undergoes enzymatic hydrolysis producing inactive metabolites that are also renally excreted. The total elimination of the drug depends on the kidney function and whenever this function is impaired monitoring of levetiracetam plasma concentrations is recommended [2,8,10,11].

Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a broad-spectrum antiepileptic drug of the phenyltriazine class, chemically unrelated to existing antiepileptic drugs [12,13]. Lamotrigine is effective against partial and secondarily generalized tonic–clonic seizures as monotherapy or adjunctive treatment [14]. Its mechanism of action seems to be the inhibition of the release of excitatory neurotransmitters like aspartate and glutamate and it is also involved in the blocking of voltage-dependent sodium channels [14,15]. Lamotrigine is used for the treatment of a wide spectrum of childhood epilepsies and it is also being prescribed for the treatment of bipolar disorders [12]. Lamotrigine is well absorbed after oral administration and approximately 55% of the drug is bound to plasma proteins. It undergoes biotransformation by hepatic N-glucuronidation and elimination via the renal route, displaying first-order linear kinetics with a slight auto induction period [15]. Wide inter-individual differences exist in serum/plasma lamotrigine levels achieved at any given dose, largely because of their pharmacokinetic interactions with concurrently prescribed anticonvulsants. A large variation has been reported in the elimination half-life of lamotrigine (an average elimination half-life is 33 h), as this can be influenced by concomitant therapy with other medications that inhibit or induce expression of hepatic drug-metabolizing enzymes [15,16]. Plasma concentrations in patients usually range from 1 to 4 μg/mL, but sometimes concentrations higher than 10 μg/mL can be observed [17]. Toxic symptoms are normally observed with peak concentrations above 20 μg/mL [18].

Several methods have been reported for the determination of levetiracetam and lamotrigine in biological fluids, separately or simultaneously with other anticonvulsants, including gas chromatography with nitrogen-phosphorus detection (GC/NPD) [8,19], with mass spectrometry (GC/MS) [20] or tandem mass spectrometry (GC/MS–MS) [21], high performance liquid chromatography (HPLC) with UV [1,8,15,22–24] or diode-array detection [4,16] and more recently tandem mass spectrometry (LC/MS–MS) [2,5,9,25–28]. To the best of our knowledge, there are only two previously published LC/MS–MS methods for the simultaneous determination of levetiracetam and lamotrigine in plasma samples [25,26]. There are two more published gas chromatography–mass spectrometric methods that determine levetiracetam alone in dog plasma and urine by GC/MS–MS [21] or lamotrigine along with carbamazepine in human serum by GC/MS [20].

The determination of antiepileptic blood concentrations is useful for the optimal drug treatment of epilepsy and during the investigation of forensic cases where antiepileptic drugs were identified. The aim of our work was to develop and validate a sensitive and specific GC/MS method for the determination of levetiracetam and lamotrigine in human whole blood. To the best of our knowledge, this is the first GC/MS method for the simultaneous assay of levetiracetam and lamotrigine in whole blood. The developed method was successfully applied during the investigation of forensic cases where these antiepileptic drugs were involved.

2. Materials and methods

2.1. Chemical and reagents

Levetiracetam (99.0%, Neuland Laboratories Limited, Hyderabad, India) was generously offered from Pharmathen (Athens, Greece). Lamotrigine with chemical purity declared 99.9% was purchased from LGC GmbH Standards (Luckenwalde, Germany). A reference standard of levetiracetam-d6 (internal standard) at a concentration of 100.0 μg/mL in methanol was purchased from Cerilliant Corporation (Round Rock, TX, USA). The solvents used (acetonitrile, dichloromethane, ethyl acetate, hexane, isopropanol and methanol) were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Analytical reagents were purchased as follows: pentafluoropropionic anhydride (PFPA) 99% and N,O-bis(trimethylsilyl)-trifluoracetamide (BSTFA) with 1% trimethylchlorsilane (TMCS) from Sigma–Aldrich (Steinheim, Germany), N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylsilylchloride (TBDMSCl), heptafluorobutyric anhydride (HFBA) 99% and trifluoroacetic acid (TFAA) 99% from Fluka (Steinheim, Germany), acetic anhydride 97% from Mallinckrodt (St. Louis, MO, USA) and pyridine 99.5% from Ferak (Berlin, Germany). In this study, four different types (Bond Elut LRC Certify, Bond Elut LRC Certify II, HF Bond Elut LRC-C18 and ABS Elut LRC-Nexus) of solid-phase extraction (SPE) columns were used and all were obtained from Agilent Technologies (Lake Forest, CA, USA). Human blood was obtained, after informed consent, from healthy donors and before its use it was screened by GC/MS for the presence of drugs.

2.2. Calibration and validation standards

Stock standard solutions of levetiracetam and lamotrigine at a concentration of 1.0 mg/mL were prepared separately by dissolving 10 mg of each compound in 10 mL of methanol and stored at −20 °C. Six working standard solutions containing both levetiracetam and lamotrigine, at the following concentrations 5.00, 10.0, 30.0, 100.0, 200.0 and 500.0 μg/mL, were prepared by mixing the appropriate volumes of the corresponding stock solutions of each compound and then by diluting with methanol. Spiked blood samples for calibration curves (calibrators) were prepared by spiking 200 μL of blank human blood with 20 μL of the mixed working standard solutions. The six calibrators contained levetiracetam and lamotrigine at equal concentrations of 0.50, 1.00, 3.00, 10.0, 20.0 and 50.0 μg/mL.

Additional working standard solutions containing levetiracetam and lamotrigine were prepared (at three different concentrations 15.0, 150.0 and 400.0 μg/mL) from different stock solutions than the ones used for calibrators, in order to prepare blood quality control (QC) samples. The three blood QC samples contained 1.50, 15.0 and 40.0 μg/mL of levetiracetam and lamotrigine, and were prepared in a similar way with the one of calibrators. Fresh working solutions were prepared on a daily basis.

A working internal standard solution containing levetiracetam-d6 at 20.0 μg/mL was prepared by diluting the appropriate volume of the corresponding stock standard solution with methanol.

Calibration curves (based on the peak area ratio of each analyte to the internal standard) were plotted every day and were used for the calculation of the analytes' concentration.

2.3. Sample preparation

A volume of 20 μL working internal standard solution of levetiracetam-d6 (20.0 μg/mL) was added to all samples and they were vortex mixed for 15 s. Therefore, all calibrators, QC and patient samples contained 2.00 μg/mL of internal standard. The pH of all