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Simultaneous determination of lamotrigine, phenobarbitone, carbamazepine and phenytoin in human serum by high-performance liquid chromatography

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Abstract

A simple reversed-phase high-performance liquid chromatography (HPLC) method was developed for the simultaneous estimation of the antiepileptic drugs (AEDs) lamotrigine (LTG), phenobarbitone (PB), carbamazepine (CBZ) and phenytoin (PHT) in human serum. The procedure involves extraction of the AEDs by mixing 200 μl of serum with 200 μl of acetonitrile containing 10 μg/ml of pentobarbitone as internal standard (IS). After centrifugation, 10 μl of the supernatant was injected onto a NOVA PAK C-18 column (250 mm × 4.6 mm, 5 μm Hypersil ODS) and eluted with a mobile phase consisting of phosphate buffer (10 mM)–methanol–acetonitrile–acetone in the ratio of 55:22:12:11 (v/v) adjusted to pH 7.0. A UV detector set at 210 nm was employed for detection. The AEDs were well resolved from the human serum constituents and the internal standard. The method can quantify LTG, PB, CBZ, and PHT at concentrations as low as 0.2 μg/ml. The method was quantitatively evaluated in terms of linearity, accuracy, precision, recovery, selectivity, sensitivity, and specificity. The method is simple, convenient, and suitable for the analysis of AEDs from human serum.

Keywords: Lamotrigine; Phenobarbitone; Carbamazepine; Phenytoin; Serum; High-performance liquid chromatography

1. Introduction

Therapeutic drug monitoring (TDM) of antiepileptic drugs is necessary to optimize the patient's clinical outcome by managing their medication regimen with the assistance of measured drug concentration [1]. Plasma concentration monitoring is widely used for the clinical management of epileptic patients receiving phenytoin (PHT), phenobarbitone (PB) and carbamazepine (CBZ) [2]. Lamotrigine (LTG), a new antiepileptic drug that is currently used as an add-on or monotherapy in patients with partial and secondary generalized seizures, also requires monitoring. There are large inter-individual variations in dose versus serum concentrations in patients on monotherapy, and pharmacokinetic variability plays a major role in the lamotrigine dosage requirements to achieve optimum serum concentrations depending on interacting AEDs comedication [3].

There are several HPLC methods reported for determination of lamotrigine in human serum or plasma [4–9] and there are various HPLC methods reported for the simultaneous determination of PHT, PB and CBZ [10–13]. For research work related to the study of drug interactions, pharmacokinetic studies and routine therapeutic drug monitoring, analytical methods that can reliably and simultaneously measure LTG and other AEDs are highly desirable. But there are very few HPLC methods for the determination of LTG simultaneously with other AEDs in plasma or serum [14–17].

Meyler et al. [14] described an HPLC method for the determination of LTG with PHT, PB and CBZ. In that method, LTG coeluted with CBZ showing broad tailing peaks. Ramachandran et al. [15] used dual wavelengths for the measurement of LTG with PHT, PB and CBZ, but they were unable to separate lamotrigine from interference with carbamazepine-

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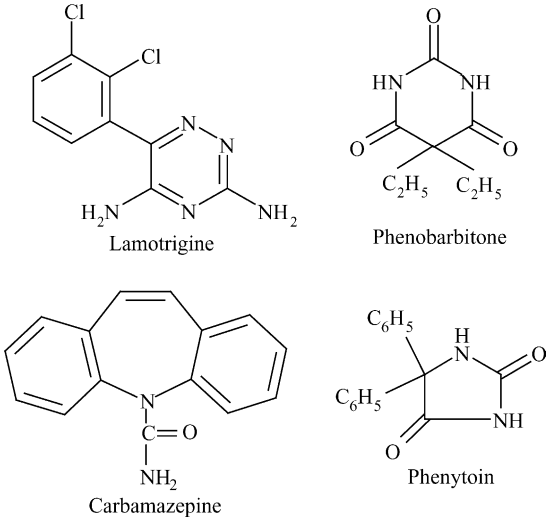

Fig. 1. Chemical structures of lamotrigine, phenobarbitone, carbamazepine, and phenytoin.

10,11-epoxide (CBZ-E). The method described by Lensmeyer et al. [16] reported the simultaneous determination of LTG with PHT, CBZ and CBZ-E but not PB, which is also a widely used AED in concomitant therapy. This method involves the solid-phase extraction of drugs.

The method described by Matar et al. [17] is the only method which can be used for the simultaneous determination of LTG, PHT, PB, CBZ and CBZ-E. This method has limitation due to the long extraction procedure and also partial overlapping of peaks (LTG with PB and PHT with CBZ) was seen, which can reduce the accuracy of the measurements.

In the present work, we report a new isocratic reversed-phase HPLC-UV method for the simultaneous measurement of lamotrigine, phenobarbitone, carbamazepine and phenytoin (Fig. 1) in human serum.

2. Materials and method

2.1. Apparatus

The high-performance liquid chromatographic system was equipped with a JASCO solvent delivery pump (PU-1580), JASCO autosampler (AS-1555), JASCO UV/visible detector (UV-1575) and Borwin Chromatographic Software for data integration, all supplied by JASCO Corporation, Tokyo, Japan. Chromatographic separations were performed using a NOVA PAK C-18 stainless steel column (250 × 4.6 mm. 5 μm Hypersil ODS) supplied by Thermo Quest Hypersil Division, Mumbai, India. The main column was protected and preceded by a guard cartridge (7.5 mm × 4.6 mm, 5 μm Hypersil ODS) supplied by Flexit Jour Pvt. Ltd., Pune, India.

2.2. Drugs and chemicals

LTG was provided as a gift sample from RPG Life Sciences Ltd., Mumbai, India, and PHT and CBZ were provided as gift samples by Sun Pharmaceuticals (Mumbai, India). PB and pentobarbitone (IS) (Sigma Chemicals) and drug-free human serum was donated by Department of Pharmacology, B J Medical College and Sassoon Hospital, Pune, India. CBZ-E was purchased from Sigma–Aldrich Inc., St. Louis, USA. Solvents used were of HPLC grade and all other chemicals and reagents were of analytical grade.

2.3. Preparation of standard solutions

A stock solution containing 1 mg/ml each of LTG, PB, CBZ and PHT was prepared in methanol. The calibration standards (0.5, 1, 5, 10, 20 and 40 μg/ml) were prepared by further dilution of stock solution with drug-free human serum. Another stock solution containing 1 mg/ml pentobarbitone (IS) was prepared in methanol and was further diluted with acetonitrile to give a concentration of 10 μg/ml. All solutions were stored at −20 °C.

2.4. Chromatographic conditions

The mobile phase consisted of phosphate buffer (10 mM)–methanol–acetonitrile–acetone (55:22:12:11, v/v/v/v) at pH adjusted to 7.0 with 0.5 M NaOH. The mobile phase was always freshly prepared and was degassed and filtered by using a Millipore vacuum filter system equipped with 0.45 μm membrane filter. The 10 mM phosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) in 1 L of doubly distilled water. Chromatography was performed at ambient temperature by pumping the mobile phase at a flow rate of 1.2 ml/min. The column effluent was monitored at 210 nm.

2.5. Extraction procedure

Serum (200 μl) was transferred to polypropylene micro tubes (1.5 ml) and 200 μl of internal standard solution was added to it. The mixture was vortex-mixed for 30 s and centrifuged at 11,000 × *g* for 20 min. The supernatant was transferred to a clean, similarly labeled tube, and recentrifuged for 5 min. The supernatant was filtered through a filtration assembly equipped with 0.2 μm membrane filter, and 10 μl of filtrate was injected onto the column.

2.6. Application

The method was used for therapeutic drug monitoring in about 200 epileptic patients presently under the treatment of different antiepileptic drugs (LTG, PB, CBZ and PHT). The study was carried out after approval of protocol from Institutional Ethical Committee of B J Medical College and Sassoon Hospital, Pune, India. Venous blood sam-