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Short communication

Simple and rapid analysis of lamotrigine, a novel antiepileptic, in human serum by high-performance liquid chromatography using a solid-phase extraction technique

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Abstract

A simple and rapid method for the quantitation of concentrations of lamotrigine, a novel antiepileptic, in human serum was developed with high-performance liquid chromatography, using a solid-phase extraction technique. The mobile phase was composed of acetonitrile–10 m*M* phosphate buffer (pH 3.5) containing 5 m*M* sodium octanesulphonate (27:73, v/v), and components were detected at 265 nm. Retention times of acetanilide as an internal standard and lamotrigine were 3.4 and 10.3 min, respectively. The coefficients of variation were 3.1–4.5% and 4.4–9.8% for the within-day and between-day precision estimates, respectively. The extraction recovery of lamotrigine added to blank serum was 86–107%. The quantitation limit of lamotrigine was ca. 0.2 μg/ml in 100 μl of serum. These results suggest that the method employed in this study is useful for the routine monitoring of sereum concentrations of lamotrigine in epileptic patients.

1. Introduction

Lamotrigine, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine (LTG, Fig. 1), is a novel antiepileptic, which has a phenytoin-like membrane-stabilizing mechanism via blockade of voltage-dependent sodium channels and inhibition of glutamate release [1]. In preclinical studies, LTG has been shown to be effective on both maximal electroshock seizures and pentylenetetrazol-evoked hindlimb extension in mice and rats [2]. The mean elimination half-life of LTG alone was 24.1 h in normal volunteers [3]. However, co-medication with carbamazepine and/or phenytoin reduced the half-life of LTG to 15 h, and co-medication with valproic acid prolonged it to 59 h in persons with epilepsy [4]. Because the combination of antiepileptics affects the plasma level of LTG, it is important to monitor plasma levels of LTG during therapy. Analytical

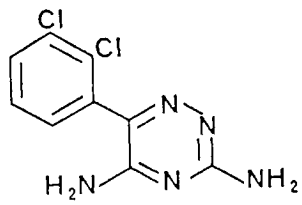

Fig. 1. Structure of lamotrigine.

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methods for the quantitation of concentrations of LTG in human plasma and serum by high-performance liquid chromatography (HPLC) [3,5,6], radioimmunoassay [7] and immunofluorometric assay [8] have been reported in connection with therapeutic monitoring in epileptic patients. However, the extraction procedures reported for HPLC were complicated (centrifugation, layer transfer and evaporation). We have measured the LTG levels of patient serum by reversed-phase HPLC using an ion-pairing agent and solid-phase extraction technique (only pour into a cartridge). The present study describes simple and rapid quantitation of LTG.

2. Experimental

2.1. Chromatographic system

The HPLC system (Waters, Milford, MA, USA) consisted of a pump (Model 510), UV detector (Model 481 Lambda-Max), autoinjector (Model 710B-WISP) and chromatogram data calculator (Model 730 Data-Module). A stainless-steel column (125 mm × 4.0 mm I.D.) packed with ODS LiChroCART C_{18} (4 μm particle size) was used at room temperature. The mobile phase was acetonitrile–0.01 *M* phosphate buffer pH 3.5, containing 5 m*M* sodium octanesulphonate (SOS) (27:73, v/v) degassed by ultrasonic vibration. The flow-rate was 1.0 ml/min. The detector was set at 265 nm, and the sensitivity was 0.02 a.u.f.s.

2.2. Reagents and standard solution

LTG was kindly donated by Nihon Wellcome (Kobe, Japan). Acetanilide as the internal standard (I.S.) was obtained from Wako Pure (Osaka, Japan). SOS, reagent grade for HPLC, was obtained from Eastman Kodak (Rochester, NY, USA). Acetonitrile and methanol, reagent grade for HPLC, were obtained from Katayama (Osaka, Japan).

A standard stock solution of LTG was prepared by dissolving 10 mg in 100 ml of methanol. This solution was diluted with methanol to obtain concentrations of 0.5, 1, 2, 5, 10, 20 and 40 μg/ml. After 1 ml of each solution was pipetted into a 10-ml test tube, the methanol solvent was evaporated at 40°C under a stream of nitrogen gas. The residue of each standard was then dissolved in 1 ml of drug-free serum and stored at −20°C until the assay. The I.S. solution was prepared at 6 μg/ml in 50% methanol solution.

2.3. Extraction apparatus

A Bond-Elut cartridge column (1 ml volume, Cat. No. 607101) and a Vac-Elut system were obtained from Analytichem International and Varian (Harbor City, CA, USA).

2.4. Procedure

The Bond-Elut cartridge column, set on the Vac-Elut chamber connected to a vacuum pump, was successively washed with 1 ml of methanol twice and 1 ml of 0.01 *M* phosphate buffer pH 3.5 containing 5 m*M* SOS twice. Then, 100 μl of serum, 50 μl of I.S. solution and 0.8 ml of 0.01 *M* phosphate buffer pH 3.5 containing 5 m*M* SOS were mixed and applied to the column. After washing with 1 ml of 0.01 *M* phosphate buffer pH 3.5 containing 5 m*M* SOS, LTG and I.S. were eluted with 250 μl of methanol. A 40-μl sample of this eluate was injected onto the HPLC system.

3. Results and discussion

We attempted to use 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BW A725C) as the I.S. However, this compound was not separated from zonisamide (Dainippon Seiyaku) by the HPLC system used in the present study. Other authors who used BW A725C as the I.S. did not report separation from zonisamide. Therefore, we used acetanilide as the I.S., which was effectively separated from zonisamide. As shown in Fig. 2, the retention times of I.S. and LTG were 3.4 and 10.3 min, respectively. No interfering endogenous substances were present. Retention times of the antiepileptic drugs ethosuximide, primidone,

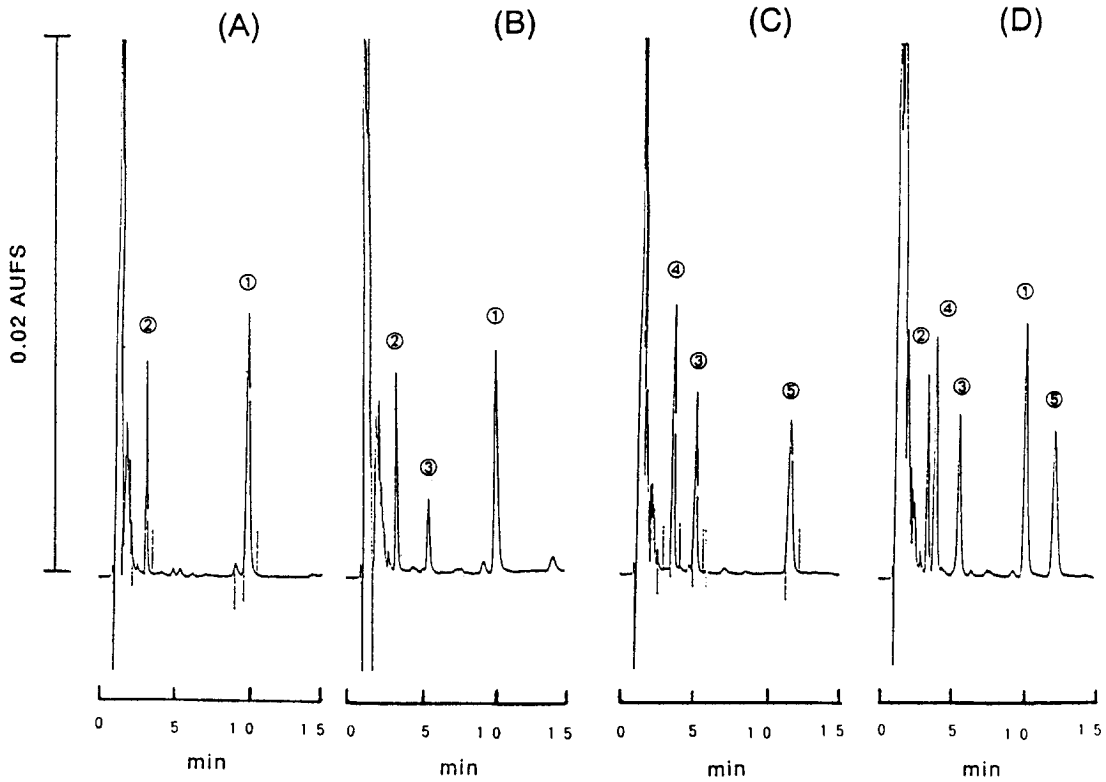

Fig. 2. Typical chromatograms of lamotrigine (LTG) and internal standard (I.S., acetanilide). (A) LTG (5 μg/ml) and I.S. (6 μg/ml) were added to drug-free blank serum. (B) Serum of a patient to whom LTG (400 mg/day) had been administered. (C) LTG-free serum of a patient to whom zonisamide (200 mg/day), primidone (400 mg/day) and carbamazepine (300 mg/day) had been administered. (D) Sample C mixed with LTG (5 μg/ml) and I.S. (6 μg/ml). Peaks: 1 = LTG; 2 = I.S.; 3 = phenobarbital; 4 = zonisamide; 5 = carbamazepine.

zonisamide, carbamazepine-10,11-epoxide, phenobarbital, carbamazepine, phenytoin and clonazepam were 2.4, 2.5, 4.0, 5.1, 5.6, 12.3, 14.3 and 19.3 min, respectively. The peaks of ethosuximide and primidone overlapped with the front peak. No valproate peak appeared during 45 min. The peaks corresponding to LTG, I.S. and other antiepileptic drugs were separated satisfactorily (Fig. 2).

An excellent linear correlation was obtained between the LTG/I.S. peak-area ratio in serum in the range 0.5–40 μg/ml LTG. The regression equation was $y = 0.654x + 0.070$, and the linear regression coefficient (r^2) was 0.998. The quantitation limit of LTG was approximately 0.2 μg/ml in 100 μl of serum. The extraction recovery, calculated by adding LTG at concentrations of 0.5, 1.0 and 2.5 μg/ml to serum, averaged 95% (86–107%).

To test the precision estimate of the assay of LTG in serum, within-day and between-day assays were performed. The within-day coefficients of variation (C.V.) of three serum samples including low, medium and high quality control (QC) concentrations in the putative therapeutic range were 4.5%, 4.5% and 3.1%, respectively (Table 1). The between-day C.V.s of three different serum samples including low, medium and high QC concentrations of LTG over eight days were 9.8%, 6.3% and 4.4%, respectively (Table 1).

The major metabolite of LTG is LTG 2-N-glucuronide (LTG-2NG). Sinz and Remmel [9] reported on the assay of LTG-2NG in blood and

Table 1
Precision of the assay of lamotrigine in serum samples

Within-day				Between-day			
Sample	*n*	Concentration		Sample	*n*	Concentration	
		(mean ± S.D.) (μg/ml)	C.V. (%)			(mean ± S.D.) (μg/ml)	C.V. (%)
A	10	0.88 ± 0.04	4.5	D	1	0.92 ± 0.09	9.8
B	10	2.02 ± 0.09	4.5	E	1	2.05 ± 0.13	6.3
C	10	4.20 ± 0.13	3.1	F	1	4.31 ± 0.19	4.4

C.V. = coefficient of variation. AD, BE and CF show low, medium and high concentrations of lamotrigine. Between-day assay run over eight days.

urine by reversed-phase HPLC. In this report, the retention times of LTG-2NG, BW A725C and LTG were 6.7, 11.9 and 19.9 min, respectively. In the present study, using reversed-phase HPLC, the retention times of acetanilide, BW A725C and LTG were 3.4, 4.1 and 10.3 min, respectively. Therefore, it was expected that acetanilide and LTG could be separated sufficiently from LTG-2NG, although a metabolic investigation of LTG was not performed.

Regarding methods for the determination of LTG in serum or plasma, liquid–liquid extraction from serum and plasma has already been tested [3,5]. However, these liquid–liquid extraction methods are limited with regard to the pretreatment of LTG , i.e. the serum and plasma extractions are complicated and there is a further need to evaporate the sample after extraction. The analytical method for the simultaneous quantitation of LTG and LTG-2NG in guinea pig using the solid-phase extraction method developed recently for simple and rapid monitoring is complicated by heating the column and graduating the percentage of the mobile phase [9]. In addition, the analytical time needed can be as long as 30 min. In the present experiment, solid-phase extraction with a Bond-Elut cartridge column containing octadecyl-bonded silica is the only pretreatment step of the HPLC method. Since this pretreatment procedure is simple and rapid, ten serum samples can be treated within 15 min. Also, the analysis time of HPLC is less than 12 min. In conclusion, the present method is useful for routine monitoring or pharmacokinetic studies of LTG concentrations in serum.

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