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Short communication

Determination of lamotrigine in human serum by liquid chromatography

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Abstract

A rapid high-performance liquid chromatographic method was developed using a short silica column (30 mm×4.6 mm) with an aqueous methanol mobile phase consisting of methanol–water–$NH_4H_2PO_4$ (94:5.96:0.04) adjusted to a final apparent pH of 5.0 and pumped at a flow-rate of 1 ml/min. Ultraviolet detection was carried out at a wavelength of 280 nm, and serum samples were prepared for HPLC analysis by extraction into dichloromethane after basification. Lamotrigine was eluted at 0.96 min. Within-day variation of the method was 4.46% at 0.75 μg/ml and 2.37% at 6.0 μg/ml, and day-to-day variation was 9.10% at 0.75 μg/ml and 7.28% at 6.0 μg/ml.

Keywords: Lamotrigine

1. Introduction

Lamotrigine (LTG) is a phenyltriazine anticonvulsant drug that is chemically and pharmacologically unrelated to currently used antiepileptic medications. It acts by inhibiting pre-synaptic voltage-sensitive sodium channels and excitatory neurotransmitter release (principally glutamate), and inhibits repetitive firing of action potentials characteristic of epileptic foci. It has been effective against refractory partial seizures, as well as generalized tonic-clonic seizures and other generalized seizures [1].

Serum LTG levels achieved with any given dose, have shown wide interindividual differences, largely because of pharmacokinetic interactions with concurrently prescribed anticonvulsants, mainly phenytoin, carbamazepine and valproate [2]. Although the value of monitoring serum LTG levels has been questioned, further studies are required to assess the relationship between concentration and clinical effect, and to evaluate changes in LTG kinetics in relation to type of comedication [1].

The chromatographic methods reported in the literature for LTG determination use conventional columns (75 to 250 mm length) with retention times between 1.5 and 10.3 min [3,4]. In the present article, we describe an isocratic normal-phase high-performance liquid chromatography (HPLC) assay for LTG in a 30 mm long column.

2. Experimental

2.1. Standards and reagents

LTG was kindly supplied by Glaxo Wellcome (London, UK). The internal standard, protriptyline

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hydrochloride, was provided by Merck Sharp&Dohme (Rahway, NJ, USA). Methanol and ammonium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany). All chromatographic solvents were of HPLC grade, and all other chemicals were of analytical grade.

2.2. Instrumentation

The analysis was performed on a Kontron chromatograph equipped with a Model 325 solvent-delivery system, a Model 465 automatic sample injector with variable-injection volume, and a Model 432 ultraviolet absorption variable-wavelength detector with an 8-μl flow cell. The detector response was monitored by an Acer 1120 SX computer with Kontron PC-integrator software, version 3.00.

2.3. Chromatographic conditions

A modification of the method described by Sallustio and Morris was used [5]. Chromatographic separation was carried out on a Perkin-Elmer silica column (30 mm×4.6 mm I.D.), with 3 μm particle size. The mobile phase was methanol–water–1 *M* $NH_4H_2PO_4$, adjusted to a final apparent pH of 5.0 with 1 *M* H_3PO_4 (94:5.96:0.04, v/v). The flow-rate was 1.0 ml/min. A sample volume of 80 μl was injected into the column. The detector wavelength was set at 280 nm and 0.02 absorbance units full scale (AUFS).

2.4. Preparation of standards for calibration curves

A stock standard LTG solution (1000 μg/ml) was prepared in methanol. Working calibrators at concentrations of 0.5, 1, 5, 10 and 20 μg/ml were prepared in drug-free serum from healthy humans by dilution. The internal standard (I.S.) was obtained by dissolving 50 mg of protriptyline hydrochloride in 50 ml of methanol, then diluting to 50 μg/ml (working standard solution). All the solutions were stored at −20°C until assayed. In order to determine whether use of an internal standard was required, calibration curves were obtained in two ways: by plotting the peak area of LTG to that of the I.S. against the nominal concentration or by calculating the peak area of the drug against its concentration.

2.5. Sample preparation

A modification of the method used by Bartoli et al. was used [2]. A 100-μl volume of I.S., 250 μl 2 *M* sodium hydroxide, and 2 ml of dichloromethane were added to a 300-μl sample in a 10-ml centrifuge tube. After mixing for 10 min, the sample was centrifuged for 5 min at 1000 *g*. The aqueous phase was discarded, and an aliquot of the clear organic phase (approximately 500 μl) was transferred to the autosampler vial. An 80-μl volume was injected, by duplicate, into the analytical column.

2.6. Analytical recovery and precision

Analytical efficiency was determined by comparing the peak area of LTG from extracted working calibrators with the peak from a direct injection of aqueous standards both at concentrations of 0.5 and 20 μg/ml.

Within-day variation was determined by assaying serum samples of known concentrations, 0.75 and 6.0 μg/ml, 15 times in the same run. Day-to-day variation was calculated by assaying serum samples of known concentrations, 0.75 and 6.0 μg/ml, once a day for 15 days, respectively.

2.7. Limit of quantification

The limit of quantification was calculated by extrapolating to concentration 0 the regression line obtained by analyzing LTG concentrations of 1, 0.5 and 0.25 μg/ml, seven times.

2.8. Drug interferences

To further confirm the accuracy of the assay, we conducted an interference study with the most common antiepileptics administered concomitantly with LTG. The following drugs were tested at their toxic concentrations: phenytoin (40 μg/ml), phenobarbital (80 μg/ml) and carbamazepine (20 μg/ml).

Table 1
Imprecision of the LTG HPLC assay with I.S. (n=15)

Concentration (μg/ml),			RSD (%)
Added	Found (x±SD)	Range	
Within-day			
0.75	0.70±0.02	0.66–0.73	3.46
6.0	5.51±0.45	4.87–5.96	8.22
Day-to-day			
0.75	0.65±0.09	0.52–0.82	13.7
6.0	5.83±0.73	4.26–7.04	12.5

Table 2
Imprecision of the LTG HPLC assay without I.S. (n=15)

Concentration (μg/ml),			RSD (%)
Added	Found (x±SD)	Range	
Within-day			
0.75	0.72±0.03	0.67–0.77	4.46
6.0	6.03±0.14	5.70–6.30	2.37
Day-to-day			
0.75	0.71±0.06	0.61–0.82	9.10
6.0	6.21±0.45	5.63–7.18	7.28

3. Results

The accuracy and precision of the method with and without the use of I.S. was calculated. Within-day and day-to-day relative standard deviations (RSDs) are presented in Tables 1 and 2.

The retention times for LTG and I.S. were 0.96 and 1.35 min, respectively. Fig. 1 shows a typical chromatogram obtained with the procedure without I.S. The limit of quantification was 0.35 μg/ml.

The day-to-day regression equation between the peak area (y) and the concentration (x) was y= 5.45x−0.5 (n=10, r=0.984). Mean recoveries from the extraction procedure were 86.7±8.9% at 0.5 μg/ml and 83.9±12.1% at 20 μg/ml (n=4).

4. Discussion and conclusions

The present method differs only slightly from those described by Bartoli et al. [2] and Sallustio and Morris [5] regarding extraction and mobile phase. The main innovation resides in the use of a short column, 30 mm long. All the chromatographic methods previously described for the determination

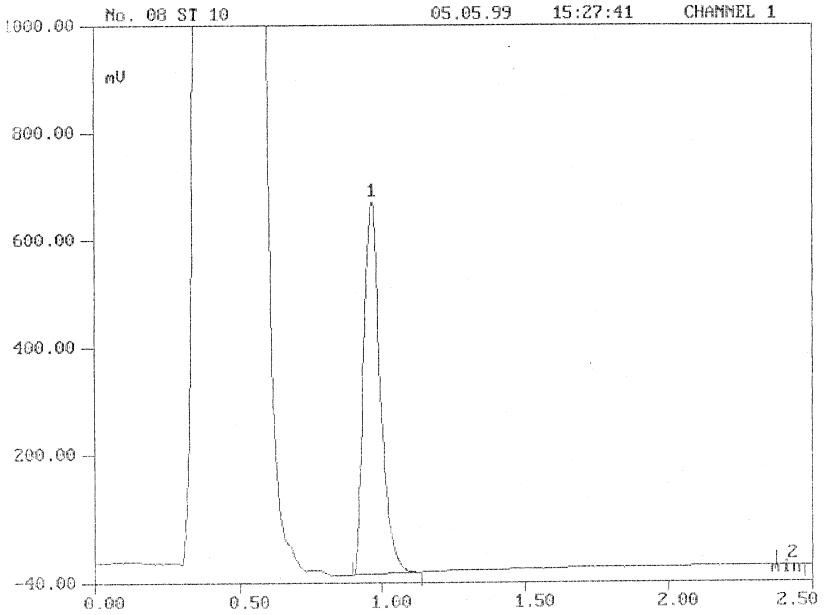

Fig. 1. Typical chromatogram of a working serum calibrator containing 5 μg/ml of LTG (1). Retention time: 0.96 min.

of LTG in biological fluids have utilized conventional columns, at least 75 mm long [6]. The use of short columns presents a series of advantages over these techniques: fast equilibrium, shorter retention times, reduced consumption of the mobile phase and the possibility to work without precolumn; all are important factors for evaluating the practicability of the method.

Liquid–liquid extraction in alkaline medium does not require evaporation of the organic phase; thus we assessed the need for use of an I.S. with the technique. Comparison of the accuracy of results with and without use of I.S. showed that mean values obtained without I.S. presented less bias than those calculated using I.S. Likewise the within-day and day-to-day run RSDs were lower without I.S. for the two concentration levels studied. Analysis time is 2.5 min per injection when working without I.S.

The quantification limit of LTG was similar to that reported by others [4]. The range of the calibration curve (from 0.5 to 20 μg/ml) covered the therapeutic interval of the drug. Although a clear relationship between LTG serum concentrations and clinical effect has not been established, a target serum range from 1 to 4 μg/ml has been proposed in earlier studies [7]. However, other investigators observed that some patients with refractory epilepsy frequently require concentrations higher than 15 μg/ml [8].

In conclusion, our results, indicate that the determination of LTG by short column normal-phase liquid chromatography is a fast and accurate method suitable for therapeutic monitoring of this drug in clinical laboratories.

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