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Journal of Chromatography B, 669 (1995) 157–162

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Determination of gabapentin in serum by capillary electrophoresis

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Abstract

A rapid capillary electrophoresis method for the quantification of gabapentin, a new anticonvulsant drug, in serum was developed. The assay involves derivatization of gabapentin with fluorescamine to provide a chromophore for UV–fluorescence detection. The migration time is about 11 min. The assay was linear between 0 and 20 mg/l. No other therapeutic drugs or amino acids interfered with the gabapentin peak. The relative standard deviation is 2.4% at a mean of 11 mg/l ($n = 17$). The mean serum level for 52 patients on this drug was 5.2 mg/l with a range of 0–12 mg/l.

1. Introduction

Gabapentin [1-(aminomethyl)cyclohexane acetic acid] is a new anticonvulsant drug, which was developed because of its structural similarity to γ-aminobutyric acid (GABA), the major neurotransmitter in the human brain [1]. Gabapentin (Neurotin) has been found to be effective as an add-on drug in the treatment of partial seizures, although its mechanism of action is unknown.

Unlike GABA, gabapentin has the ability to cross the blood–brain barrier. It has very low toxicity in humans, and it is well absorbed. The drug is not metabolized, rather it is mostly excreted unchanged in the urine, and it shows no appreciable protein-binding [2]. The elimination half-life ($t_{1/2}$) of gabapentin is approximately 5–7 h [2]. The therapeutic window has not been well established; however, patients who respond to this drug had serum gabapentin concentrations of >2 mg/l [3].

In pharmacokinetic investigations, gabapentin has been assayed by an HPLC procedure [4] involving a pre-column derivatization of the drug with 2,4,6-trinitrobenzenesulphonic acid (TNBS) followed by UV detection, and by a GC procedure involving a pretreatment with an ion-exchange resin column [5]. Both procedures are very laborious involving organic extractions, evaporation to dryness, reconstitution and derivatization.

Here we present a simple and relatively rapid capillary electrophoresis (CE) method for the quantification of gabapentin in serum. The procedure is based on derivatization of the drug with fluorescamine and protein precipitation all

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0378-4347/95/$09.50
SSDI 0378-4347(95)00081-X

in one step, followed by injection on the capillary.

2. Experimental

2.1. Reagents

Gabapentin (Neurotin) was obtained from Parke-Davis (Morris Plains, NJ, USA), fluorescamine from Sigma (St. Louis, MO, USA), and boric acid from Fisher (Fairlawn, NJ, USA).

2.2. Instrumentation

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA, USA) was set at 35 μA, 24°C, and 200 nm. The capillary was 60 cm × 50 μm I.D.. The electrophoresis buffer was a mixture of boric acid (200 mmol/l) and potassium phosphate dibasic (26 mmol/l). Samples were introduced by pressure injection for 15 s. Each morning, the capillary was conditioned by rinsing for 7 min with sodium hydroxide (2 mol/l) and for 7 min with the electrophoresis buffer.

2.3. Procedure

To 50 μl of serum samples or standard in 1.5-ml Eppendorf tubes were added 200 μl of acetonitrile containing fluorescamine (2.5 mg/ml of acetonitrile prepared daily). The samples were vortex-mixed thoroughly for 60 s and then centrifuged for 30 s at 14 000 *g*. The supernatant was introduced into the capillary. The samples were electrophoresed for 12 min, and after each run the capillary was washed for 2 min with NaOH (2 mol/l) and the electrophoresis buffer. The reacted samples were found to be stable for about 12 min; however, as acetonitrile evaporates rapidly, they were analyzed within 3 min after preparation.

3. Results and discussion

Attachment of a chromophoric group to gabapentin facilitates and increases the sensitivity of its detection. Fluorescamine was chosen as the derivatizing reagent because of its fast reactivity (few seconds), and formation of a chromophoric group that is detectable both by UV and fluorescence. Although we used UV absorbance at 200 nm in this work, fluorescence detection, when it is available, offers a better sensitivity and selectivity. We were able to combine the fluorescamine derivatization and deproteinization in one step by dissolving the fluorescamine in acetonitrile. The narrow serum pH (7.4) provides the necessary buffer for the reaction too. It has been previously shown that deproteinization by acetonitrile is a suitable method for removal of serum proteins for drug determination by CE [6].

Due to the structural similarities of gabapentin and the naturally occurring amino acids, one of the major concerns of the procedure is the susceptibility to interference by some of the endogenous amino acids present in the serum samples. By properly selecting the optimum analysis conditions of buffer, voltage, and capillary length, gabapentin is separated from the many interfering peaks as seen Fig. 1. The migration time for gabapentin was about 11 min. The gabapentin peak was verified in patient samples by spiking (Fig 2). Analysis of a spiked and unspiked sample containing a standard solu-

Table 1
List of drugs tested for interference

Drug	Concentration (mg/l)
Acetaminophen	4.0
Carbazepine	8.7
Disopyramide	3.8
Gentamicin	5.5
Lidocaine	4.5
N-Acetylprocainamide	4.8
Phenytoin	15
Primidone	5.7
Procainamide	8.0
Quinidine	3.7
Salicylate	150
Theophylline	15
Tobramycin	4.6
Valproic acid	77
Vancomycin	19

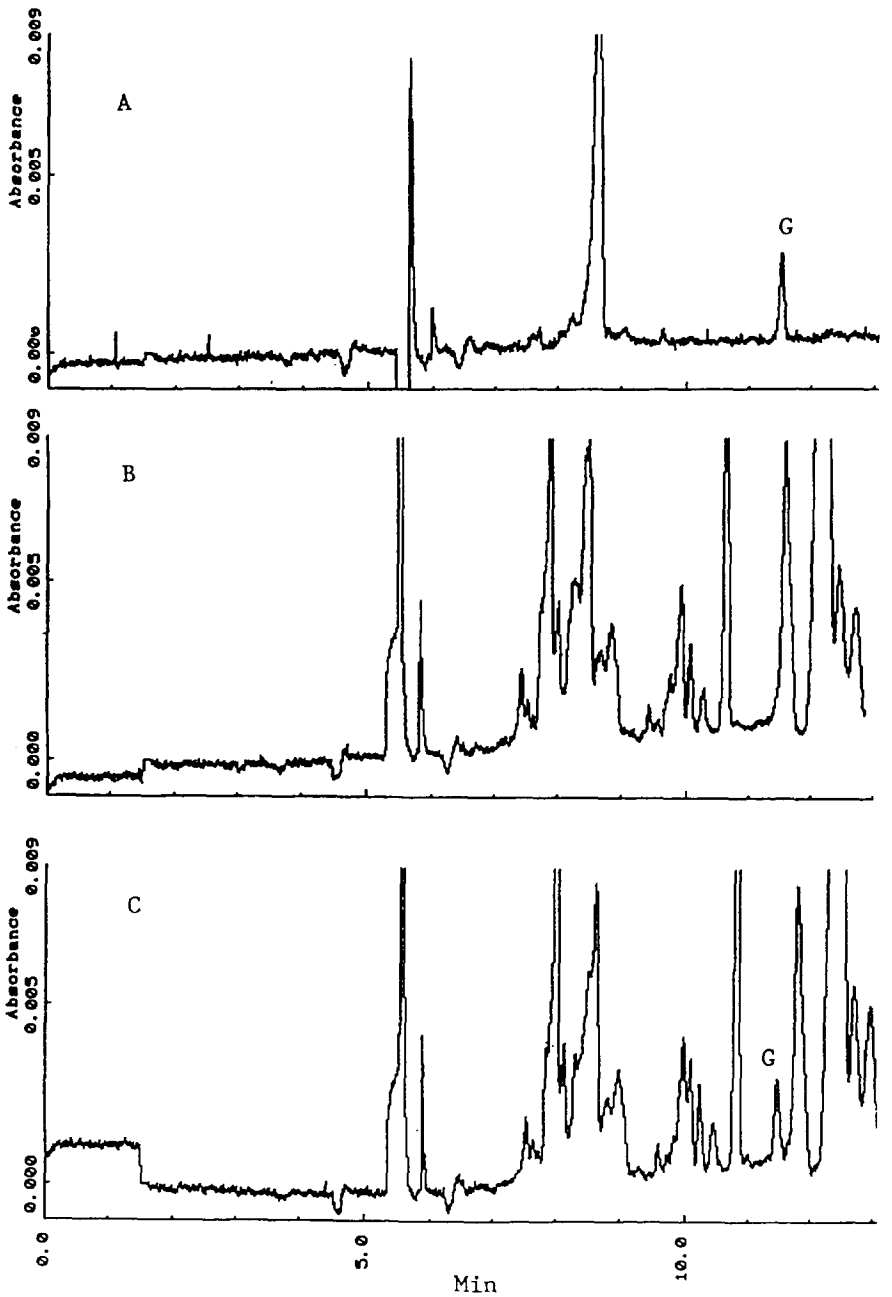

Fig. 1. Electropherogram of (A) gabapentin (G) added to water, 6.5 mg/l; (B) serum free of gabapentin; and (C) the serum of (B) with gabapentin added, 6.5 mg/l.

tion of 23 amino acids including GABA, showed no interference with the gabapentin peak. The same result was obtained when a sample containing the common drugs listed in Table 1 was analyzed spiked and unspiked with gabapentin. Furthermore, no interference was observed when five different serum pools were analyzed spiked and unspiked.

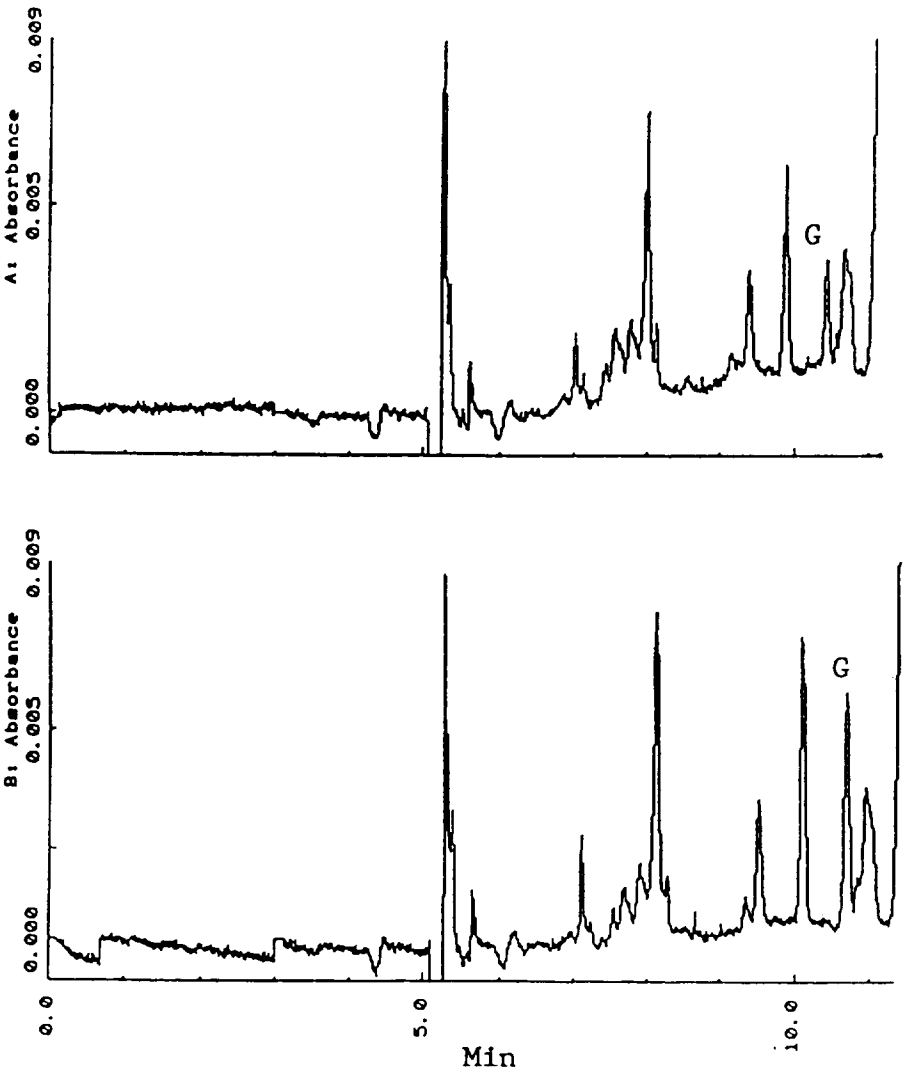

Fig. 2. Electropherogram of (top) a patient on gabapentin (G), 4.9 mg/l; and (bottom) the same patient after spiking with gabapentin, 6.0 mg /l.

A standard serum curve in the range of 1 to 20 mg/l of gabapentin shows linearity, as measured by peak height, throughout this range, which is over the clinically relevant concentration (mA = Conc. $\times$ 0.255 – 0.051; $r = 0.99$). Peak height was used here instead of area because it does not require electronic integration. The minimum detectable concentration was determined to be approximately 1 mg/l in serum. A good mixing or sonication of the sample with the fluorescamine in addition to a good rinse of the capillary with sodium hydroxide after each run were important for good reproducibility. Occasionally, the migration time changed suddenly and the capillary required a long wash as described in section 2.2. The capillary at least can be used for 100 injections. The separation in general was similar on different capillaries with slight differences in the migration time of gabapentin. The within-day relative standard deviation was 2.4%

($n = 17$, mean 11 mg/l). The average recovery of gabapentin from serum was 92.7% relative to that from aqueous solution ($n = 3$). To avoid the problem of a low recovery we prepared the standard in serum.

Four serum samples were analyzed on the first day (2.3, 5.3, 9.1 and 10.7 mg/l) and five days later after storage at 4°C (1.9, 5.5, 9.3 and 11.1 mg/l). Fig. 3 illustrates the analysis of a sample on the first day and after five days, using different batches of buffer and fluorescamine. Although the peaks for some of the amino acids changed after storage, gabapentin did not. Based on these data, it seems that gabapentin in the serum remains stable for at least five days when stored at 4°C

We analyzed the sera of 52 patients on this drug. The mean serum level of gabapentin was 5.2 ± 2.1 mg/l, with a range of 0 to 12 mg/l. Two patients with elevated values of 10 and 12 mg/l were tolerating the drug well without any side effects. In conclusion, this method for determination of serum gabapentin using capillary electrophoresis is simple and rapid as opposed to other HPLC and GC methods that are time-consuming, expensive and labor-intensive.

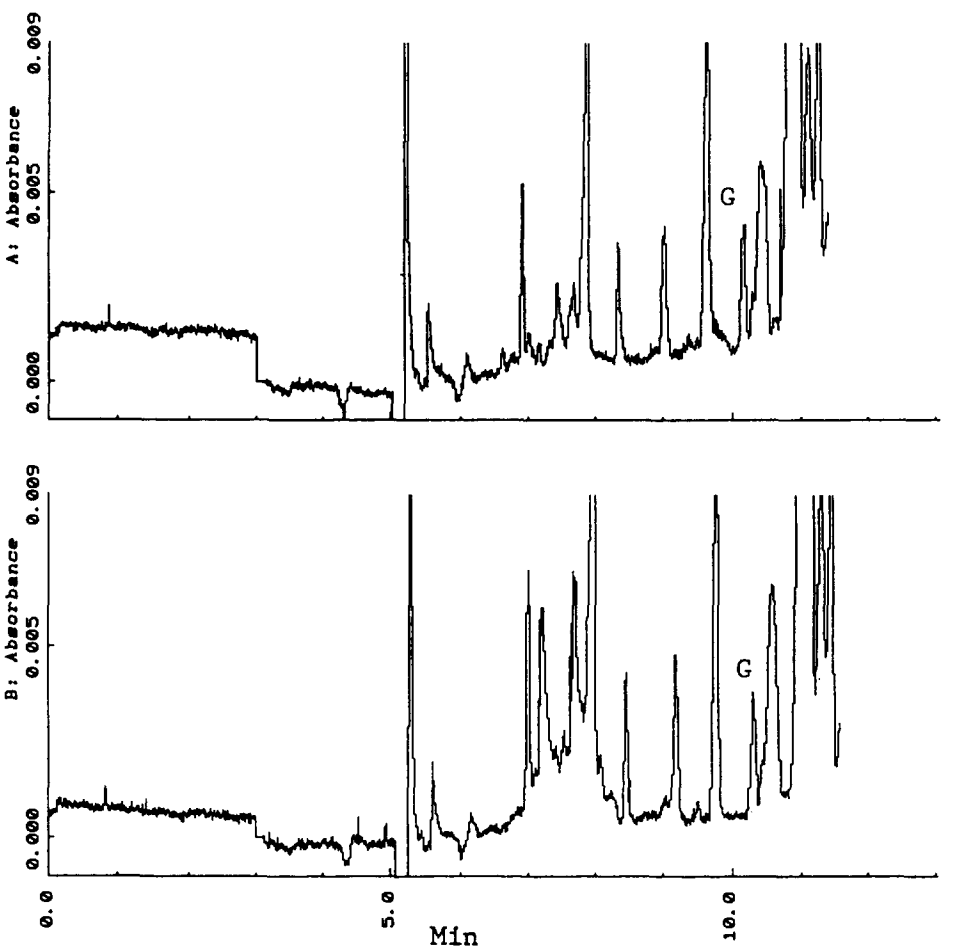

Fig. 3. Stability of gabapentin (G) in a patient. (A) The sample reacted with fluorescamine and analyzed on the first day; and (B) after 5 days of storage at 4°C, reacted again with a different batch of fluorescamine and analyzed.

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