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Rapid high-performance liquid chromatographic determination of serum gabapentin

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Abstract

Gabapentin (GBP) is a new antiepileptic drug approved for clinical treatment of partial seizures in the USA. Serum GBP concentrations in 283 patients were studied using high-performance liquid chromatography with fluorescence detection. The standard curves were linear over a range of 60 ng to 15 μg/ml. The coefficient of variations were 3.4 to 8.8% and 1.4 to 9.8% for intra- and inter-assay studies, respectively. The lower limit of quantitation was 10 ng/ml. Of the 283 patients studied, 72.5% had GBP levels between 2 and 10 μg/ml, 14.8% were below 2 μg/ml and 12.7% above 10 μg/ml. The mean±S.E. of GBP in 283 patients was 5.38±0.23 μg/ml. Peak concentrations of more than 15 μg/ml and trough levels as low as 0.1 μg/ml were not uncommon. The method described was rapid, simple, highly sensitive and reproducible. Other antiepileptic drugs and endogenous compounds did not interfere with the assay.

Keywords: Gabapentin

1. Introduction

Gabapentin (GBP) is a new antiepileptic drug used clinically for the treatment of partial onset seizures with or without secondary generalized tonic–clonic convulsions. Although it has a chemical structure related to γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, it does not interact with GABA receptors. It is neither a GABA agonist nor an inhibitor of GABA uptake or degradation [1]. Pharmacokinetically, GBP is known for its rapid absorption after oral administration with a maximum blood concentration occurring in 2 to 3 h. The absorption is dose-dependent and saturable [2,3]. The drug has little protein-binding capability. With a half-life of 5 to 7 h, GBP is eliminated in the urine with a clearance proportional to that of creatinine. However, the action mechanism of GBP is still unknown. Today GBP is widely accepted clinically. Sensitive and reliable assays to measure its blood concentrations at both high and low levels are required for furthering studies of its efficacy, dose-related side effects and action mechanism.

Of the several published methods [1–8], the quantitation of GBP using high-performance liquid chromatography (HPLC) with either UV or fluorescence detection was the preferred method [3,6,8]. However, complicated extractions, long run time and lower sensitivity have hampered its use in clinical drug monitoring [3]. The method described here is a modification of the one reported by Forrest et al. [3].

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By using a Supelco LC-8DS column, automated pre-column derivatization and other modified conditions, we were able to achieve much shorter run times and higher sensitivity. These were of great significance for our detecting both peak and trough GBP levels and managing high sample volumes. This assay has been successfully used in our study of 283 patients with partial onset epilepsy treated with GBP at routinely prescribed doses.

2. Experimental

2.1. Reagents

Pure GBP, 1-(aminomethyl)cyclohexane acetic acid, and internal standard, 1-(aminomethyl) cycloheptane acetic acid, were provided by Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI, USA). Positive controls of GBP (levels I and II) were purchased from Utak Labs. (Valencia, CA, USA). Phthalaldehyde (OPA), 3-mercapto-propionic acid (MPA) and boric acid were obtained from Aldrich (Milwaukee, WI, USA). Human drug-free serum was purchased from Bio-Rad (Chicago, IL, USA). EDTA disodium salt and sodium hydroxide were bought from Sigma (St. Louis, MO, USA). Other reagents including methanol, perchloric acid, glacial acetic acid and HPLC-grade water were all from Fisher Scientific (Tustin, CA, USA).

2.2. Standards and solutions

Stock solutions of GBP and internal standard were prepared in HPLC-grade water (1 mg/ml) and stored at −20°C for six months. Working solutions of GBP were made by diluting the stock with drug-free sera (60 ng to 15 μg/ml). Working solution of internal standard was diluted from the stock by using HPLC-grade water (50 μg/ml). Borate buffer (0.5 *M*, pH 9.5) was prepared by dissolving 30.91 g of boric acid in 500 ml water and using sodium hydroxide to adjust pH. Perchloric acid (4 *M*) was diluted from its commercial concentrate (70%). The fluorescent solution of OPA–MPA was made daily by mixing 50 mg OPA with 4.5 ml methanol, 0.5 ml borate buffer and 50 μl MPA. The EDTA–acetate buffer (pH 3.7) was composed of EDTA (0.1 g/l) and glacial acetic acid (18 ml/l).

2.3. Patients

Two hundred eighty three patient serum samples were sent from other hospitals or clinics in the USA. The specimens were shipped to our laboratory at 4°C or frozen in dry ice. Of the 283 patients treated with GBP at routinely prescribed doses, 15 were children under 15 years of age, 214 were adults and 54 were over 60 years old. More detailed diagnostic and treatment information about the patients were not available.

2.4. Apparatus

The study was performed on a Beckman HPLC system (Fullerton, CA, USA) equipped with Model 126 Beckman HPLC pumps, Model 406 interface, Model L-7480 Hitachi fluorescence detector and Beckman Gold system software running on an IBM personal computer. A Model SIL-10A Shimadzu autosampler and system controller (Columbia, MD, USA) were used for automatic injections.

2.5. Chromatographic conditions

Separations were carried out at room temperature on Supelco LC-8DS analytical column (Supelco, Bellefonte, PA, USA) with a Bio-Rad guard column, serving as stationary phase. The mobile phase was isocratic, consisting of EDTA–acetate buffer (pH 3.7)–methanol–acetonitrile (40:30:30, v/v), and maintained at 2 ml/min. Detection wavelengths were set at 320 and 450 nm for excitation and emission. Run time was 8 min per sample.

2.6. Sample preparation

Standard curves were prepared by adding 200 μl GBP working solutions, at concentrations of 60 ng, 180 ng, 540 ng, 1.6 μg, 5 μg and 15 μg, and 20 μl working internal standard into microcentrifuge tubes. Two GBP commercial controls (3 and 12 μg/ml) and patient samples were transferred to their respective tubes using 200 μl sera and 20 μl internal standard. Deproteinization was carried out by adding

20 μl of 4 *M* perchloric acid to every tube, followed by vortex-mixing for 10 s and centrifuging 2 min at 17 225 *g* at room temperature. The supernatant (25 μl), methanol (100 μl) and borate buffer (100 μl) were added into conical glass inserts. After mixing by shaking manually, the inserts were put into amber bottles, which were then loaded to the autosampler. The Shimadzu system controller was programed to add 25 μl OPA–MPA and mix for 2 min. A waiting period of 4 min was allowed to complete the automatic derivatization process and 10 μl was injected into the HPLC system by the autosampler.

2.7. Calculation

A standard curve was made by calculating the linear regression of the amounts of GBP versus the peak area ratios of GBP over the internal standard obtained in the assay. The concentrations of GBP in experimental samples were determined by their peak area ratios of GBP over the internal standard and the regression coefficient of the curve. A Student *t*-test was used for comparing some GBP concentrations initially obtained and the ones acquired after samples were stored at 4°C for one month.

3. Results

Typical chromatograms of GBP are shown in Fig. 1: (A) drug-free serum only, (B) drug-free serum spiked with 3 μg/ml GBP and 1 μg internal standard, (C) serum from a patient spiked with 1 μg of internal standard before derivatization. The chromatograms clearly show that GBP and internal standard were separated and free from interference by serum endogenous components. Under the conditions described for the test, GBP and internal standard were eluted at 4.9 and 6.2 min, respectively.

Standard curves were linear over the range of 60 ng to 15 μg GBP. The correlation coefficient of the standard curves varied from 0.996 to 0.999. The reproducibility of the method was studied over a period of six days by assaying three pooled drug-free sera spiked with GBP. The results of this study are shown in Table 1. The coefficients of variations (CVs) ranged from 3.4 to 8.8% for intra-assay and 1.4 to 9.8% for inter-assay determinations. The recovery of GBP from sera was 100%. This was estimated by studying the peak area ratios of GBP in sera and spiked reference over internal standard. The limit of quantitation was 60 ng/ml. A lower limit of 10 ng/ml was achieved when 50 μl sample was injected into the autosampler.

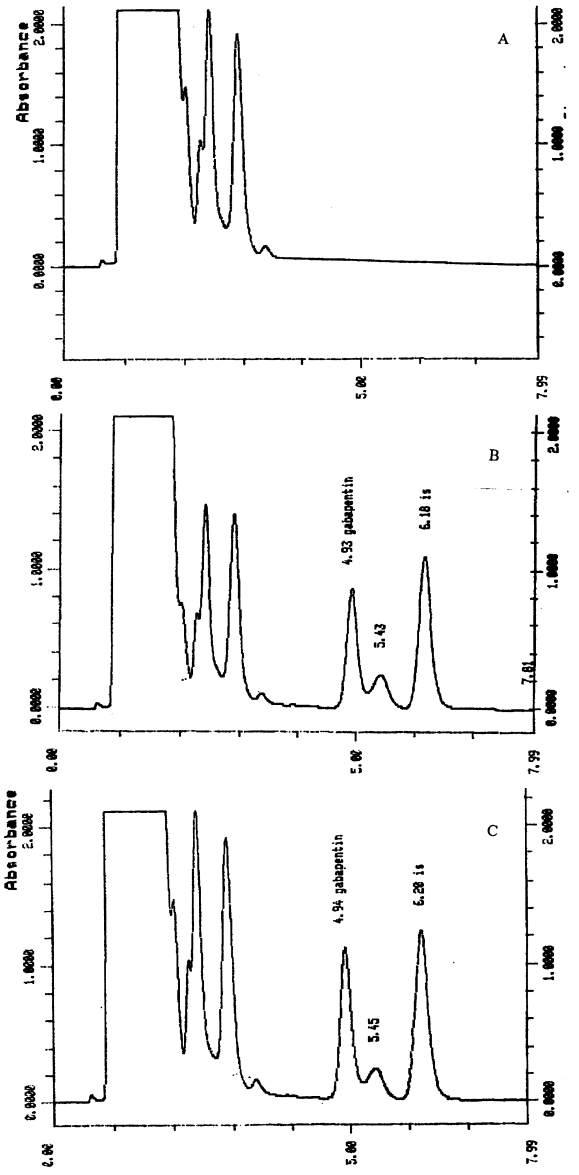

Fig. 1. Chromatograms of serum gabapentin determination: (A) drug-free serum; (B) drug-free serum with spiked GBP (3 μg/ml) and internal standard (1 μg); (C) patient serum with spiked internal standard (1 μg).

Table 1
Reproducibility of serum gabapentin assay

Added gabapentin (μg/ml)	Intra-assay (n=6)			Inter-assay (n=6)		
	Mean (μg/ml)	CV[a] (%)	Accuracy (%)	Mean (μg/ml)	CV (%)	Accuracy (%)
0.15	0.14	8.8	93.3	0.15	9.8	100
3.5	3.4	6.7	97.1	3.1	7.7	88.6
12.5	12.6	3.4	99.2	12.2	1.4	97.6

[a] CV=Coefficient of variation.

Table 2 compares the initial GBP concentration and the value obtained from the same samples one month later. The samples were stored at 4°C between assays. The Student t-test showed that there was no statistically significant difference between the means of the two groups (t=1.62, P=0.13), suggesting that GBP be stable at least for one month at 4°C.

Table 3 illustrates the blood GBP levels in 283 patients treated with GBP for partial onset seizures. The GBP concentrations were 2 to 10 μg/ml in 195 (72.5%), below 2 μg/ml in 52 (14.8%), and above 10 μg/ml in 36 (12.7%) patients. The mean±standard error (S.E.) was 6.80±1.16, 5.48±0.27 and 4.55±0.49 μg/ml for children, adults and elderly, respectively. The mean±S.E. for 283 patients as a whole was 5.38±0.23 μg/ml.

Table 2
Repeated serum gabapentin assay after one month[a]

Sample No.	First assay (μg/ml)	Second assay (μg/ml)
1	2.1	2.0
2	4.4	5.7
3	8.3	7.5
4	5.2	3.8
5	0.5	0.3
6	3.5	3.0
7	2.4	2.2
8	0.8	0.8
9	3.6	3.8
10	4.4	3.9
11	3.9	3.2
12	1.7	1.4
13	5.7	4.1
14	3.1	3.5
Mean	3.5	3.2

[a] Student t-test: t=1.6215, P=0.1289.

We also included phenytoin, phenobarbital, benzodiazepines, carbamazepine, primidone, vigabatrin and lamotrine in our interference study. These drugs either did not show up in the chromatogram or were eluted early with other compounds. There was no interference peak at the same retention times of GBP and the internal standard under the conditions described for this assay.

4. Discussion

Although several reports of GBP determination using HPLC methods have been published in the literature, significant drawbacks prevent them from being widely used in clinical laboratories for therapeutic drug monitoring [3]. Lensmeyer et al. [2] reported an optimized method for determination of GBP in serum, which used C_{18} solid-phase extraction, trinitrobenzene derivatization and C_{18} solid-phase membrane concentration to improve chromatographic selectivity. They were able to achieve 15 min run time and 50 ng/ml detection limit although they had a multistep procedure of time-consuming

Table 3
Serum gabapentin concentrations of 283 patients

	By age of patient (years)			By gabapentin level (μg/ml)			
	<15	15–60	>60	0.1–1.9	2.0–10.0	10.0–20.0	>20.0
Mean (μg/ml)	6.80	5.48	4.55	0.90	5.13	13.1	5.38
Standard error	1.16	0.27	0.48	0.08	0.16	0.34	0.23
Size (%)	15 (5.3)	214 (75.6)	54 (19.1)	52 (14.8)	195 (72.5)	36 (12.7)	283 (100)

sample preparations. Forrest et al. [3] preferred one-step sample derivatization and fluorescence detection for their HPLC methodology in quantification of plasma GBP. Wad and Kramer [8] also used HPLC with fluorescence detection for their study. The method we described here was a modification of the one reported by Forrest et al. By using a Supelco LC-8DS column, automatic one-step pre-column derivatization and other modified chromatographic conditions, we obtained a 60 ng/ml quantitation limit that could be further decreased to 10 ng/ml when more samples were injected in contrast to 500 ng/ml obtained by Forrest et al. and 0.5 μmol/l by Wad and Kramer. The run time per sample in our test was much shorter than the assay times reported by other authors using HPLC with fluorescence detection.

In current clinical practice, the routine doses of GBP administered by doctors may vary according to patient conditions and whether other antiepileptic drugs are also used. The reported therapeutic range of GBP is 2 to 10 μg/ml [2,3]. In our studies of 283 patients, 195 (72.5%) fell into this range. However, some patients reached as high as 17.8 μg/ml that exceeded the highest level of standard curve. We had to use half the amount of serum for them. Also, some patients had trough GBP levels as low as 0.1 μg/ml. As seen in Table 3, the mean±S.E. of the 52 patients with serum GBP below 2 μg/ml was 0.9±0.08. Although this group only accounted for 14.8% of total patient numbers in this study, the determinations of the low drug levels were surely important to both clinicians and patients regarding clinical pharmacokinetics, drug efficacy and side-effects.

As noticed by other investigators [3,8], the fully-derivatized GBP products degraded easily about 25 min after derivatization. In our study, GBP itself was stable for at least one month at 4°C although OPA–MPA solution degraded in 24 h even without derivatization. We not only made fresh OPA–MPA on a daily basis but also used an autosampler to add into vials and mix well before injection. To assure no contamination, the autosampler needle was washed thoroughly between injections. Since the derivatization was automatically carried out during a run and the run time was as short as 8 min, more than 150 samples could theoretically be processed daily in our laboratory and the possibility of OPA–MPA degradation after derivatization was reduced to a minimum.

5. Conclusions

Serum GBP concentrations in 283 patients were studied using HPLC with fluorescence detection. GBP levels were between 2 and 10 μg/ml in 72.5%, below 2 μg/ml in 14.8% and above 10 μg/ml in 12.7% patients. The mean±S.E. of GBP in 283 patients was 5.38±0.23 μg/ml. The peak values of more than 15 μg/ml and trough levels as low as 0.1 μg/ml were not uncommon. The method described was rapid, simple, highly sensitive and reproducible. Other antiepileptic drugs and endogenous compounds did not interfere with our test. The assay has been used successfully in our study to determine the serum GBP concentrations in patients treated with clinically prescribed GBP at routine doses.

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