ELSEVIER

Journal of Chromatography B, 705 (1998) 154–158

JOURNAL OF CHROMATOGRAPHY B

Short communication

Sensitive high-performance liquid chromatographic method with fluorometric detection for the simultaneous determination of gabapentin and vigabatrin in serum and urine

N. Wad*, G. Krämer

Swiss Epilepsy Centre, Bleulerstrasse 60, CH-8008 Zürich, Switzerland

Received 28 January 1997; received in revised form 7 October 1997; accepted 16 October 1997

Abstract

Serum concentrations of the antiepileptic drug gabapentin (GBP) are usually determined by high-performance liquid chromatography (HPLC) using UV photometric detection after pre-column derivatization with 2,4,6-trinitrobenzenesulphonic acid. Vigabatrin levels in serum are determined by HPLC using fluorescence detection. Like vigabatrin (VGB), gabapentin has also a primary amine group that easily reacts with *o*-phthaldialdehyde reagent and produces a fluorescing substance. By the use of fluorometric detection, GBP can be determined more simply, sensitively and simultaneously with VGB. The day-to-day coefficient of variation for the determination of GBP in a pooled serum was 4.0% ($n=17$; serum concentration, 13.8 μmol/l) and for VGB was 3.1% ($n=21$; serum concentration, 26.4 μmol/l). The lower limit of detection is 0.5 μmol/l for both drugs and the method is linear up to 500 μmol/l for GBP and 1300 μmol/l for VGB.

Keywords: Gabapentin; Vigabatrin

1. Introduction

Gabapentin (GBP) [1] and vigabatrin (VGB) [2] are new antiepileptic drugs currently being introduced in therapy worldwide. Whereas VGB is structurally and functionally related to γ-amino-*n*-butyric acid (GABA), the mode of action of GBP, which is also structurally related to GABA, is still unknown. At present, VGB serum concentrations are useful in assessing compliance and evaluating risks of toxicity. Serum concentrations of GBP have not been studied to the degree of VGB, it is, however, expected that the reasons for measuring the concentration of GBP in serum will be the same. Serum concentrations of GBP are usually determined by high-performance liquid chromatography (HPLC) using UV photometric detection after pre-column derivatization with 2,4,6-trinitrobenzenesulphonic acid [3,4]. VGB levels in serum are determined by HPLC using fluorescence detection [5], a method originally developed for the determination of amino acids [6]. Like VGB, GBP also has a primary amine group that easily reacts with the *o*-phthaldialdehyde reagent and produces a fluorescing substance. By the use of fluorometric detection, GBP can be determined more simply, sensitively and simultaneously with VGB. The method is a further development of the method of Tsanaclis et al. [5] for the determi-

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00521-5

nation of VGB. Recently, Juergens et al. [7] published a method using fluorescence detection for the determination of GBP in serum. The advantages of the method presented in this paper in comparison to that of Juergens et al. [7] are included in the discussion.

2. Experimental

2.1. Reagents and standards

Acetonitrile, ethanol and methanol are LiChrosolv reagents from Merck (Darmstadt, Germany). Aldrich (Switzerland) provided us with *o*-phthaldialdehyde and 2-mercaptoethanol. VGB and the internal standard (γ-phenyl GABA) were kindly supplied by Marion Merrell Dow Research Institute (Cincinnati, OH, USA) and GBP was supplied by Gödecke Research Institute (Freiburg, Germany). The standard solution was prepared by dissolving an appropriate amount of GBP and VGB in drug-free serum. This serum standard was calibrated with an appropriate standard of GBP and VGB dissolved in 70% methanol in water.

2.2. Apparatus

Diluter: Hamilton Microlab M (Bonaduz, Switzerland). Liquid chromatograph: Hewlett-Packard

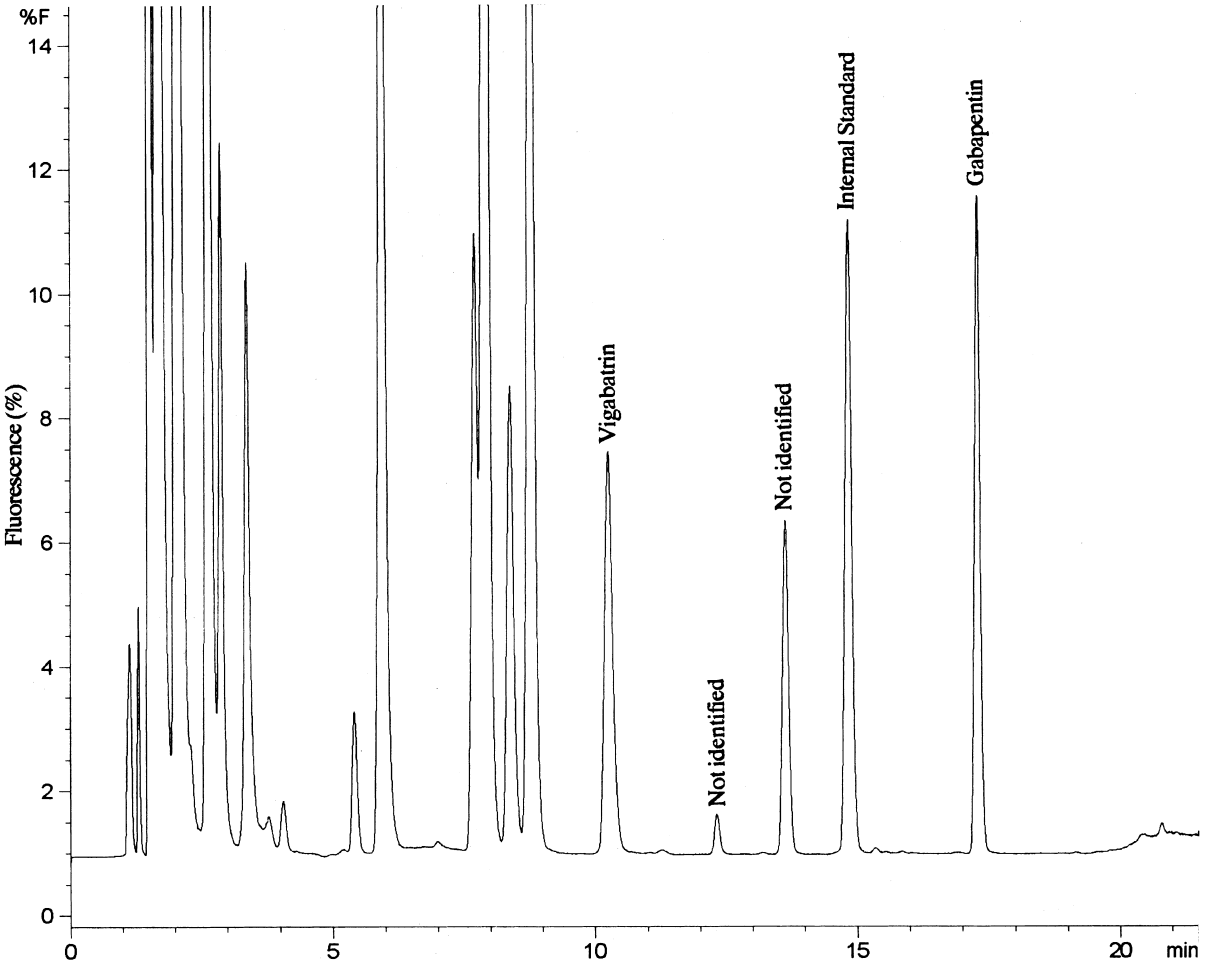

Fig. 1. Chromatographic separation of VGB (69.9 μmol/l) and GBP (36.4 μmol/l), which were added to drug-free serum and extracted as a patient serum.

Model 1090 (Waldbronn, Germany), equipped with a Hewlett-Packard 1046 A fluorescence detector. Column: Superspher 60 RP-Select B, particle size 5 μm, 125×3 mm I.D. (Merck).

2.3. Method

Sample preparation as described by Tsanaclis et al. [5] was modified to allow the use of a diluter. A 50-μl volume of serum (urine was diluted between 1:50 and 1:200 with distilled water, depending on its concentration) was added to 1000 μl of methanol containing the internal standard (3.4 mg of γ-phenyl GABA in 1000 ml of methanol). The mixture was vortex-mixed for 15 s and then centrifuged at 2000 *g* for 10 min. The protein-free supernatant was transferred to an autosampler glass vial. A 6-μl volume of the supernatant was drawn into the injector, together with 3 μl of *o*-phthaldialdehyde reagent [5], and mixed in four cycles by moving the plunger of the injector back and forth for 2 min, which was controlled automatically by the injector program in the Hewlett-Packard 1090 before the chromatographic run starts. Due to the instability of the reaction product [6], it is convenient to have a programmable injector, but it is also possible to perform this step manually before injection.

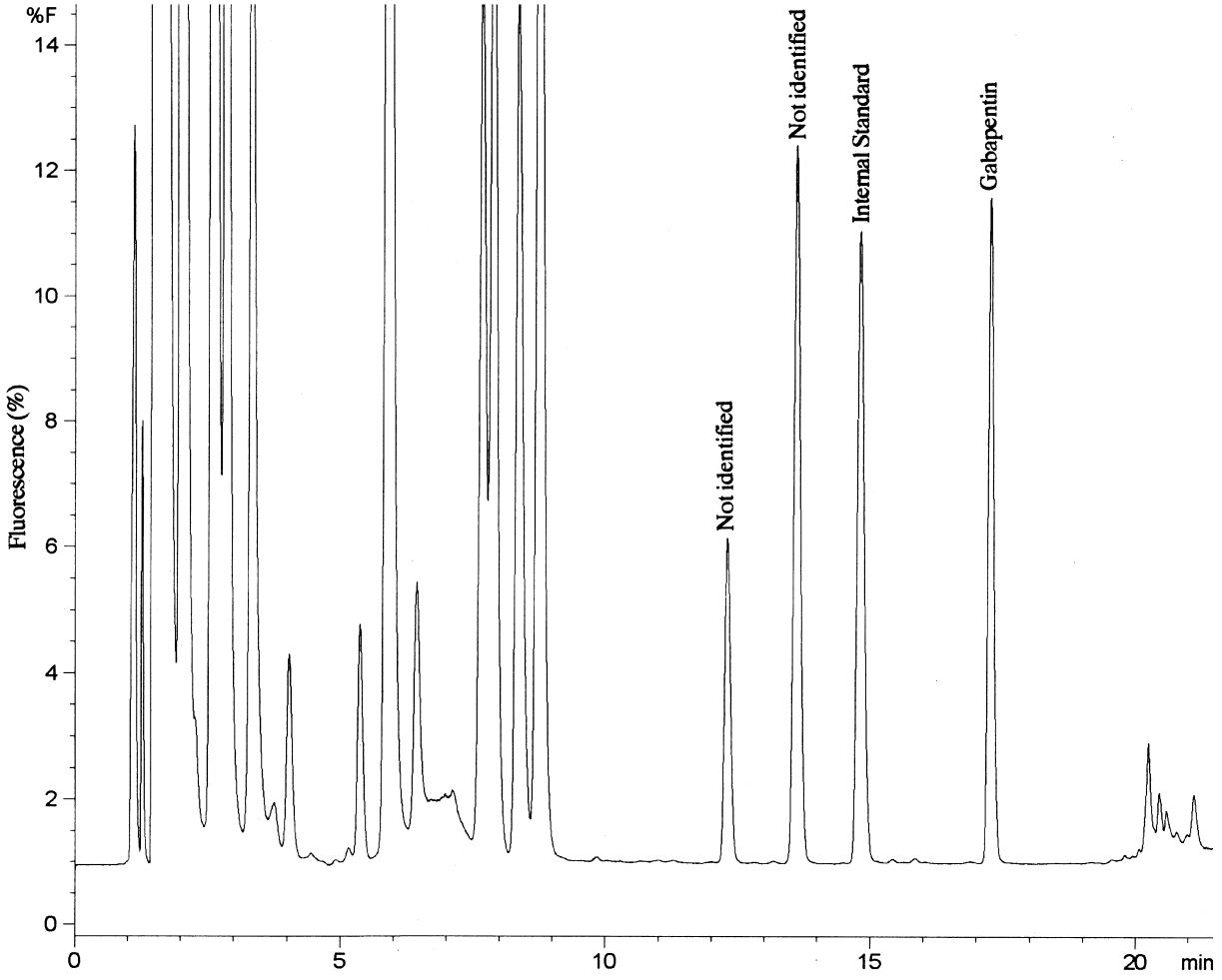

Fig. 2. Chromatographic separation of a serum extract from a patient receiving 1200 mg/day GBP and 1200 mg/day carbamazepine (CBZ). The serum concentration of GBP was 37.2 μmol/l.

2.4. Liquid chromatography

The mobile phase for the gradient elution of GBP and VGB was acetonitrile in 20 m*M* KH_2PO_4. The elution gradient went from 22 to 37% in 12 min, and finally to 55% in 6 min. The gradient was ended by a 2-min wash program with 80% acetonitrile. The total time was 21.5 min. The flow-rate was 0.7 ml/min, the temperature was 35°C and the column effluents were detected at an excitation wavelength of 230 nm and an emission wavelength of 455 nm.

3. Results and discussion

The within-day coefficient of variation for the determination of GBP in a patient's serum was 1.2% (n=16; serum concentration, 59.6 μmol/l) and 0.6% for VGB (n=16; serum concentration, 103.9 μmol/l). The within-day coefficient for GBP in a sample of urine, diluted 1:200 with distilled water, was 1.3% (n=16; 24.9 μmol/l) and in a urine sample containing VGB that was diluted 1:50, it was 1.7% (n=16; 35.2 μmol/l). The day-to-day coefficient of variation in a pooled serum was 4.0% (n=17; concentration, 13.8 μmol/l) for GBP and 3.1% (n=21; concentration, 26.4 μmol/l) for VGB. Recovery of GBP and VGB added to serum was 100%. Fig. 1 shows a chromatographic separation of GBP and VGB that were added to drug-free serum and extracted as a patient serum. GBP and VGB are well separated from the amino acids and other compounds, giving a fluorescing substance with *o*-phthaldialdehyde. These substances were eluted with

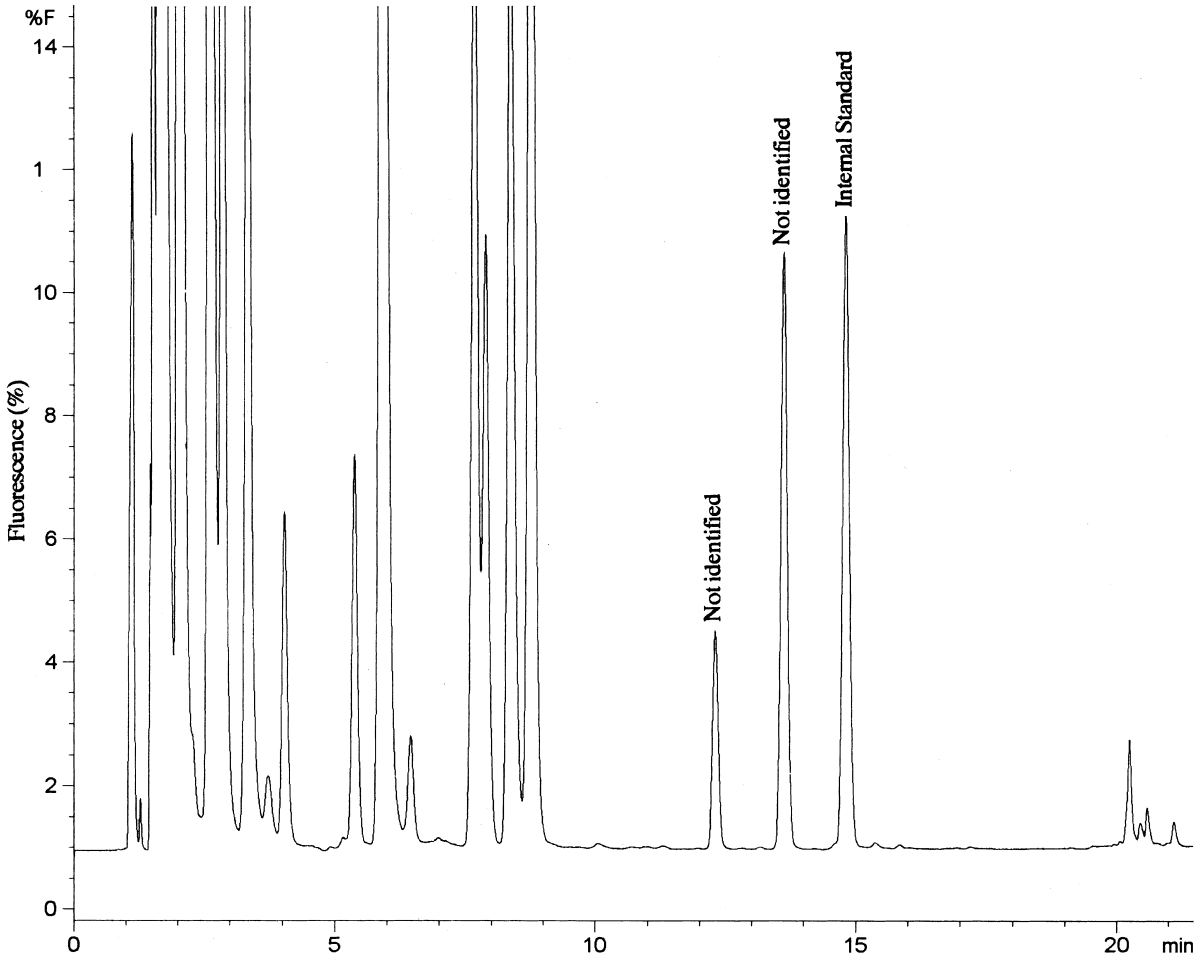

Fig. 3. Chromatographic separation of a serum extract from a patient receiving 1600 mg/day CBZ and 100 mg/day phenobarbital.

retention times between 0 and 9 min, except for two unidentified, naturally occurring substances in serum with retention times of 12.3 and 13.6 min. No interactions from other antiepileptic drugs in use were observed in serum or in urine, due to the specific fluorometric reaction. The lower limit of detection at a signal-to-noise ratio of three was 0.5 μmol/l for both drugs. The method was linear up to 500 μmol/l for GBP and 1300 μmol/l for VGB. Fig. 2 shows a chromatographic separation of a serum extract from a patient receiving GBP (1200 mg/day) and carbamazepine (1200 mg/day). The serum concentration of GBP was 37.2 μmol/l.

Fig. 3 shows a serum extract from a patient receiving 1600 mg of carbamazepine and 100 mg of phenobarbital. No other serum substances appear at the retention times of GBP and VGB.

Our method differs from that of Juergens et al. [7] in the following ways: (1) less serum is used; 50 μl compared to 500 μl; (2) the extraction time (using vortex-mixing) is 15 s instead of 5 min; (3) gradient elution involves the use of two solutions instead of three; (4) the duration of the chromatographic run is 21.5 min instead of 33.5 min; (5) the protein precipitation reagent, methanol, is less toxic than acetonitrile. In addition, with our mobile phase, we have not observed any decrease in intensity of the fluorescent signal due to re-uptake of oxygen during a HPLC sequence, as described by Juergens et al. [7].

The possibility of a simultaneous determination of GBP and VGB may be of clinical relevance as both drugs are currently used as add-on therapy in refractory partial seizures.

References

[1] A. Beydoun, B.M. Uthman, J.C. Sackellarer, Clin. Neuropharmacol. 18 (1995) 469–481.
[2] A.H. Kurland, T.R. Browne, Clin. Neuropharmacol. 17 (1994) 560–568.
[3] H. Hengy, E.U. Kölle, J. Chromatogr. 341 (1985) 473–478.
[4] G.L. Lensmeyer, T. Kempf, B.E. Gidal, D.A. Wiebe, Ther. Drug Monit. 17 (1995) 251–258.
[5] L.M. Tsanaclis, J. Wicks, J. Williams, A. Richens, Ther. Drug Monit. 13 (1991) 251–253.
[6] R.F. Chen, C. Scott, E. Trepman, Biochim. Biophys. Acta 576 (1979) 440–455.
[7] U.H. Juergens, T.W. May, B. Rambeck, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1459–1471.