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Short communication

Fast isocratic high-performance liquid chromatographic assay method for the simultaneous determination of gabapentin and vigabatrin in human serum

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1. Introduction

Gabapentin (GBA) and vigabatrin (VGA) are potent antiepileptic drugs (AEDs) that are currently being introduced in therapy worldwide. Both drugs are structural analogues of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). VGA is a chiral molecule, commercialized as the racemate, but only the *S*(+)-enantiomer is pharmacologically active [1]. *R*(−)-VGA does not undergo chiral inversion and does not interfere with the action of *S*(+)-VGA [2]. Data in the literature suggest a linear correlation with a mean (*R*)/(*S*) ratio of 1.3 [2,3].

Several HPLC assay methods have been reported for the determination of either GBA only [4–6], racemic VGA only [7–9], or VGA enantiomers only [3], in serum samples. Most of these procedures are based on the same approach, involving a simple automated *o*-phthaldialdehyde (OPA) derivatization of chemically deproteinized serum samples, which was primarily widely applied to amino-acid analysis, followed by HPLC separation in acidic mobile phases and fluorimetric detection. Because both VGA and GBA react and elute as the numerous endogenous amino acids present in serum, all procedures reported for the simultaneous determination of both VGA and GBA [10–12] require gradient-elution programs. As a consequence, chromatographic run times ranging from 22 to 35 min were required.

This paper describes HPLC conditions allowing the fast, simultaneous determination of OPA derivatives of both drugs and the internal standard within 10 min, in isocratic mode as an alternative to the gradient program methods previously reported for the simultaneous determination of serum GBA and VGA.

2. Experimental

2.1. Chemicals

GBA and its cycloheptyl derivative, (1-aminomethyl-cycloheptyl)-acetic acid, which was used as internal standard (I.S.), were kindly supplied by Parke Davis (Ann Arbor, MI, USA). Racemic VGA

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was kindly supply by Hoechst Marion Roussel (Cincinatti, OH, USA). The other reference AEDs and metabolites were obtained from either proprietary companies or commercial suppliers. Acetonitrile and methanol were of HPLC grade from Rathburn (Walkerbun, UK). Water was purified by means of a Milli-Q Plus device (Millipore, Le Mont sur Lausanne, Switzerland). All other chemicals were of analytical grade from Fluka (Buchs, Switzerland). Suitable GBA and VGA stock and working solutions were prepared in acetonitrile–water (1:1, v/v). The I.S. solution (40 μg/ml) was also prepared in acetonitrile–water (1:1, v/v). Drug-free serum was obtained from healthy volunteers and blood donors.

2.2. Instrumentation and conditions

The HPLC system consisted in a LaChrom Model L7100 pump, a LaChrom Model L7250 autosampler, a LaChrom Model L7480 fluorescence detector set at λ_{exc}=235 nm and λ_{em}=435 nm and a LaChrom Model L7000 data station, all from MerckHitachi (Merck ABS, Geneva, Switzerland). The pump was equipped with a Gastorr degassing unit from Omnilab (Chavannes de Bogis, Switzerland). A Nucleosil C_{18} 100-5 column (250×3.0 mm I.D., average diameter of solid particules (*dp*)=5 μm) from Macherey-Nagel (Oensingen, Switzerland) was used. The mobile phase was a mixture of 0.022 *M* phosphoric acid (pH 2.0)–acetonitrile (45:55, v/v). The flow-rate was 0.60 ml/min. The precolumn derivatization, the control of the HPLC system, the calibration curves (linear regression on nominal concentrations versus peak-height ratio of GBA and VGA to I.S.) and the calculations of concentration were performed automatically by means of the LaChrom system.

2.3. Sample preparation

Internal standard solution (100 μl) was added to a 300-μl serum sample placed in a regular, 1.5-ml HPLC glass vial. The sample was deproteinized using 1200 μl of acetonitrile. The capped vials were vortex-mixed and centrifuged (1000 *g* for 4 min at 20°C). The vials were placed in the autosampler rack, where they were processed by the autosampler.

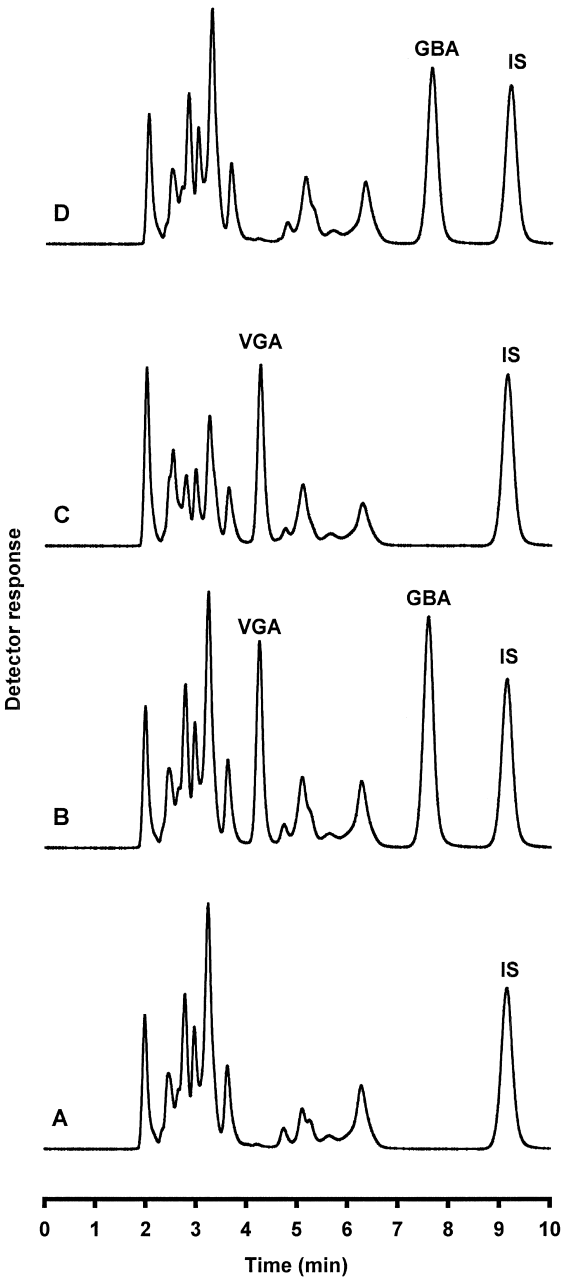

Fig. 1. Typical chromatograms of a drug-free serum sample (A), a drug-free serum sample spiked with GBA (11.22 μg/ml) and VGA (22.40 μg/ml) (B), and authentic serum samples from patients under VGA therapy (C; calculated concentration. 19.44 μg/ml) and GBA therapy (D; calculated concentration, 9.43 μg/ml). The retention times were 4.25, 7.60 and 9.15 min for VGA, GBA and I.S., respectively.

The OPA derivatization step was fully automated and took place in another HPLC vial placed in the same autosampler rack. Briefly, 150 μl of supernatant were transferred to an empty, uncapped, HPLC vial. Sodium borate buffer solution [2.0% (w/v) adjusted to pH 9.50 with 2 *M* sodium hydroxide (150 μl)] was added, followed by 150 μl of a derivatization solution that had been freshly prepared, containing 1.0% (w/v) OPA and 1.0% (v/v) mercaptoethanol in methanol–sodium borate buffer (2.0%, w/v, pH 9.50; 9:1, v/v). Mixing was performed by aspirating and dispensing the reaction mixture several times at high speed in the vial. After a 200-s reaction time, an aliquot of 2 μl was immediately injected into the HPLC system. The next sample was prepared while the previous sample was being analyzed on the analytical column.

3. Results and discussion

The isocratic HPLC conditions developed allowed the separation of GBA, VGA and I.S. from endogenous and reaction peaks within 10 min, under isocratic elution conditions. Typical chromatograms are given in Fig. 1. A possible degradation of the OPA derivatives was prevented by the automated approach applied, which involved the immediate injection of the derivatives formed. The chromatographic separation of a sample and the preparation of the next one were performed concomitantly in order to allow high sample-throughput.

The assay precision and accuracy data are given in Table 1. The repeatability, reproducibility and the accuracy of the assay were found to be suitable for its intended use. The sensitivity and linearity of the assay covered the therapeutic ranges of 1.0–26.0 μg/ml and 2.0–40.0 μg/ml for GBA and VGA, respectively. The limit of detection ($S/N=3$) was found to be in the order of 0.1 μg/ml for GBA and 0.6 μg/ml for VGA. The limit of quantification was set to the lowest concentration level of the therapeutic range, i.e., 1.0 μg/ml for GBA (precision, 3.1%; accuracy, −9.1%) and 2.0 μg/ml for GBA (precision, 3.4%; accuracy, −7.2%). The precision and the accuracy at the limits of quantification applied were lower than the acceptable limits of ±20%. No interference from endogenous compounds or com-

Table 1
Precision and accuracy of the assay for the simultaneous determination of GBA and VGA[a]

Concentration					Deviation
Nominal (μg/ml)	Found mean (μg/ml)	SD	RSD (%)	Confid. int. ($P=95\%$, $n=6$)	From nominal concentration (%)
Intra-day precision and accuracy of GBA assay					
1.01	0.92	0.03	3.3	0.03	−8.9
10.14	9.46	0.20	2.1	0.21	−6.7
20.08	19.00	0.31	1.6	0.33	−5.4
Inter-day precision and accuracy of GBA assay					
1.01	0.98	0.06	6.1	0.07	−3.0
10.14	10.18	0.25	2.5	0.26	0.4
20.08	20.86	0.74	3.5	0.78	3.9
Intra-day precision and accuracy of VGA assay					
1.96	1.82	0.06	3.3	0.06	−7.1
19.65	19.73	0.29	1.5	0.30	0.4
38.54	41.99	0.90	2.1	0.95	9.0
Inter-day precision and accuracy of VGA assay					
1.96	2.03	0.19	9.4	0.20	3.6
19.65	19.43	0.32	1.6	0.34	−1.1
38.54	40.31	0.92	2.3	0.97	4.6

[a] Abbreviations: RSD, relative standard deviation; Confid. int., confidence interval of the mean.

monly prescribed AEDs and their metabolites, such as carbamazepine, carbamazepine-10,11-epoxide, carbamazepine-*trans*-diol, monohydroxy- 10-carbamazepine, lamotrigine, phenytoin and its metabolites *m*-HPPH and *p*-HPPH, primidone and its metabolite PEMA, phenobarbital, ethosuximide, pheneturide, felbamate, topiramate, nitrazepam, clobazam, clophazine, 534U87 and its N-oxide metabolite, 2329U90, was observed. GBA and VGA were found to be stable in the supernatant of deproteinized serum stored in the autosampler rack for at least 5 h. The deviations measured for GBA after this time period were found to range from 0.1 to 3.0% at concentrations of 0.98, 10.18 and 20.86 μg/ml. For VGA levels of 10.08 μg/ml ($n=9$), the mean deviation after this time period was found to be 1.9±1.4%. GBA and VGA were found to be stable in serum frozen at −20°C for at least 4 months. The deviations measured after this time period were found to range from −4.8 to 2.0% at 0.98, 10.18 and 20.86 μg/ml, and from −2.6 to 5.4% at 2.3, 19.43 and 40.31 μg/ml, respectively.

The described assay method was successfully applied to the monitoring of VGA and GBA in epileptic patients. Concentrations ranging from 2.54 to 42.53 μg/ml (mean±SD=23.07±12.85 μg/ml; $n=54$) and from 2.43 to 22.66 μg/ml (mean±SD= 9.40±4.71 μg/ml; $n=62$) were measured for VGA and GBA, respectively. The assays were ordered only when all necessary data (e.g., daily dose the patient was actually administrated, blood sample collection time in relation to drug administration, demographic data, patient compliance) were obtained. However, only the sample identification number was available to the analysts who performed the assay as blind samples. Possible evaluation of the drug concentrations found is therefore difficult.

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