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Simultaneous HPLC-F analysis of three recent antiepileptic drugs in human plasma

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ABSTRACT

An original high-performance liquid chromatographic method with fluorescence detection is presented for the simultaneous determination of the three antiepileptic drugs gabapentin, vigabatrin and topiramate in human plasma. After pre-column derivatisation with dansyl chloride, the analytes were separated on a Hydro-RP column with a mobile phase composed of phosphate buffer (55%) and acetonitrile (45%) and detected at λ_{em} = 500 nm, exciting at 300 nm. An original pre-treatment procedure on biological samples, based on solid-phase extraction with MCX cartridges for gabapentin and vigabatrin, and with Plexa® cartridges for topiramate, gave high extraction yields (>91%), satisfactory precision (RSD < 6.4%) and good selectivity. Linearity was found in the 0.2–50.0 μg mL^{-1} range for gabapentin, in the 1.0–100.0 μg mL^{-1} range for vigabatrin and in the 1.0–50.0 μg mL^{-1} range for topiramate, with limits of detection (LODs) between 0.1 and 0.3 μg mL^{-1}. After validation, the method was successfully applied to some plasma samples from patients undergoing therapy with one or more of these drugs. Accuracy results were satisfactory (recovery >91%). Therefore, the method seems to be suitable for the therapeutic drug monitoring (TDM) of patients treated with gabapentin, vigabatrin and topiramate.

1. Introduction

Since the introduction of potassium bromide and paraldehyde (1850–1880), several generations of antiepileptic agents have been introduced into the clinical usage, greatly improving the life quality of many people suffering from seizures. Gabapentin (1-(aminomethyl)-cyclohexaneacetic acid, GBP, Fig. 1a), vigabatrin (4-amino-5-hexenoic acid, VGB, Fig. 1b) and topiramate (2,3:4,5-bis-*O*-(1-methylethylidene)-D-fructopyranose sulfamate, TPR, Fig. 1c) are three relatively recent drugs used in monotherapy and in polypharmacy for the treatment of different forms of epilepsy [1]. They are also often used as mood stabilisers in the therapy of several psychiatric disorders such as schizophrenia and bipolar disorder [2]. These antiepileptic drugs have different, and still partially unknown, mechanisms of action. While gabapentin is thought to act on voltage-dependent calcium channels, topiramate seems to act as a sodium channel blocker and a chloride channel activator [3]. Vigabatrin, on the other hand, is an irreversible suicide inhibitor of γ-aminobutyric acid (GABA) transaminase [3]. They are generally considered safer than other, older drugs, while also being equally effective. Unfortunately, even when using the most recent agents, as much as 20% of the patients is still non-responder to the therapy. Furthermore, most patients (up to 80%) experience side effects during the treatment, but should nonetheless continue it for their entire lives to obtain a sufficient control of the symptoms. It is not uncommon for patients to report hyperactivity, confusion, insomnia, nervousness, depression and even psychotic symptoms during therapy with VGB, GBP or TPR, and all of these drugs have some teratogenic potential [4]. In addition, GBP can cause hepatotoxicity and is known to be carcinogenic for some animal species [5], while up to 50% of the patients treated with VGB can show atrophy of the retinal nerve and TPR can cause vision loss (glaucoma and myopia), osteoporosis, hyperthermia and nephrolithiasis.

To effectively optimise the treatment outcome and reduce the incidence of side effects, drug doses and scheduling should be personalised. A reliable therapeutic drug monitoring (TDM) regimen should be established, especially when the patient is subjected to polypharmacy [6,7,8]. This allows the personalisation and optimisation of the therapy, minimising side and toxic effects, increasing the efficacy of the treatment and reducing its costs due to fewer hospitalisations and a more rational use of drugs and resources. However, the first step in this chain of decisions is always the development and implementation of reliable analytical methods, which

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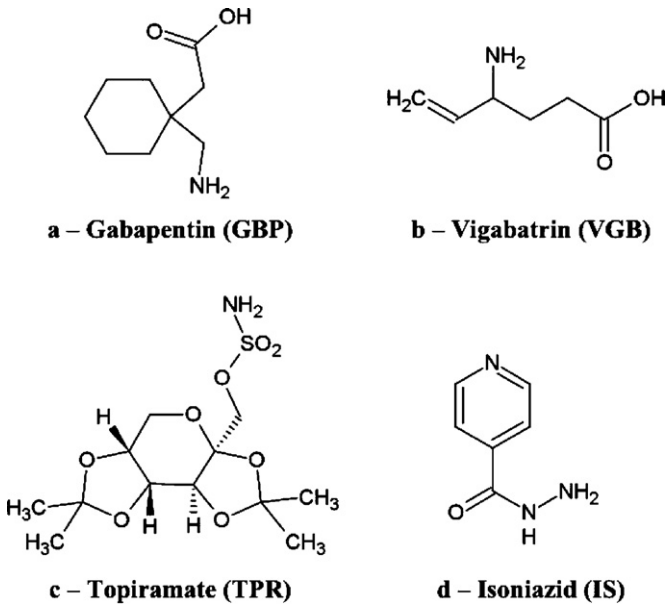

Fig. 1. Chemical structures of (a) gabapentin (GBP), (b) vigabatrin (VGB), (c) topiramate (TPR) and (d) the internal standard isoniazid (IS).

should be suitable for the repeated determination of drug plasma levels over long periods of time. For this reason, an original HPLC-F method, based on pre-column derivatisation with dansyl chloride (DC), has been developed for the analysis of GBP, VGB and TPR in patients' plasma. Several papers can be found in the literature for the analysis of GBP [9–12], VGB [13–15] or TPR [16,17]; a few other methods analyse GBP and VGB together [18–21], but none of them carries out the simultaneous analysis of all three compounds. Most methods include a derivatisation procedure to reach the desired sensitivity, e.g. with 4-chloro-7-nitrobenzofurazan [16], *o*-phthaldialdehyde [20] or dansyl chloride [21].

With respect to the method proposed herein, the HPLC method, which also used DC as a derivatising agent [21], only analyses GBP and TPR and not TPR. Moreover, it uses a low-efficiency chromatographic column (10-μm particles) and carries out the sample pre-treatment by protein precipitation, which is more prone to interference than other, more recent procedures, such as solid-phase extraction (SPE). Moreover, the whole technique is time-consuming: the chromatographic run requires 14 min for the separation of two analytes and the derivatisation procedure lasts 20 min.

An available alternative is the coupling to different detection means, such as mass spectrometry [18,19], however, this kind of instrumentation is very expensive and not available in every laboratory.

The method proposed herein has the advantage of being fast, feasible and inexpensive while granting good reliability, thus being promising for the TDM of epileptic and psychiatric patients undergoing treatment with GBP, VGB or TRP.

2. Experimental

2.1. Chemicals and solutions

GBP was kindly provided by Pfizer Inc. (New York, NY, USA) and VGB was kindly donated by Ovation Pharmaceuticals Inc. (Deerfield, IL, USA). TPR, isoniazid (pyridine-4-carbohydrazide, Fig. 1d), used as the Internal Standard (IS), acetonitrile and methanol HPLC grade, ammonia (25%, w/w), hydrochloric acid (37%, w/w), potassium phosphate bibasic, sodium carbonate and dansyl chloride (DC), all pure for analysis, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Phosphoric acid (85%, w/w) was purchased from Fluka (Buchs, Switzerland). Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, USA).

Stock solutions of the analytes and the IS (1 mg mL^{-1} each) were prepared by dissolving suitable amounts of each pure substance in methanol. Standard solutions were obtained by diluting stock solutions with the mobile phase and were subjected to the derivatisation procedure before directly injected into the HPLC. Stock solutions were stable for at least 2 months when stored at −20 °C (as assessed by HPLC assays); standard solutions were prepared fresh every day and were kept shielded from light. DC solutions (2 mg mL^{-1}) were prepared in acetonitrile.

2.2. Instrumentation and chromatographic conditions

The chromatographic system was composed of a Varian (Walnut Creek, USA) model 9001 chromatographic pump and a Varian 9075 spectrofluorimetric detector set at λ_{exc} = 300 nm, λ_{em} = 500 nm.

Separations were obtained on a Phenomenex (Torrance, CA, USA) Synergy Hydro-RP (150 mm × 4.6 mm ID, 4 μm) column. The mobile phase was composed of acetonitrile–phosphate buffer (50 mM) (45:55, v/v) (pH* 5.3), filtered through a Phenomenex membrane filter (47 mm membrane, 0.2 μm, NY) and degassed by an ultrasonic bath. A flow rate program was used as follows: 0.0–4.7 min, constant 1.0 mL min^{-1} flow rate; 4.8–5.2 min, linear gradient 1.0–2.5 mL min^{-1}; 5.3–12.0 min, constant 2.5 mL min^{-1} flow rate; 12.1–12.5 min, linear gradient 2.5–1.0 mL min^{-1}. The injections were carried out through a 50-μL loop. Data processing was handled by means of a Varian (Walnut Creek, USA) Star Chromatography 4.0 software.

Solid-phase extraction (SPE) was carried out by means of a VacElut (Varian) apparatus. A Crison (Barcelona, Spain) Basic 20 pHmeter and a Hettich (Tuttlingen, Germany) Universal 32 R centrifuge were used.

2.3. Sample collection and preparation

The blood samples were collected from epileptic and psychiatric patients admitted to the Department of Neurosciences (University of Parma, Italy) subjected to monotherapy or polypharmacy with GBP, VGB and/or TPR for at least 2 weeks at constant daily doses. Blood samples were usually drawn 12 h after the last drug administration. Blood was stored in glass tubes containing EDTA as the anticoagulant, then centrifuged (within 2 h from collection) at 1400 × *g* for 15 min; the supernatant (plasma) was then transferred into polypropylene test tubes and stored at −20 °C until HPLC analysis. "Blank" plasma was obtained in the same way from blood drawn from healthy volunteers not subjected to any pharmacological treatment.

Samples containing GBP and VGB were subjected to solid-phase extraction on Waters (Milford, MA, USA) Oasis® mixed-mode reversed-phase strong cation exchange (MCX) cartridges (30 mg, 1 mL).

MCX cartridges were conditioned with 1 mL of methanol twice and equilibrated with 1 mL of ultrapure water twice. To 500 μL of plasma, 1 mL of 0.1 N HCl and 50 μL of IS standard solution were added and the resulting mixture loaded onto a conditioned cartridge. The cartridge was then washed twice with 1 mL of 0.1 N HCl, twice with a 50 mM, pH 5.0 phosphate buffer and once with 50 μL of methanol. The analytes were then eluted with 2 mL of ammonia–water–acetonitrile (5:13:82, w/w/v). The eluate was brought to dryness, re-dissolved with 100 μL of ultrapure water and subjected to the derivatisation procedure.