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

Laura Mercolini [a], Roberto Mandrioli [a], Mario Amore [b], Maria Augusta Raggi [a,*]

[a] Faculty of Pharmacy, Department of Pharmaceutical Sciences, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy
[b] Department of Neurosciences, Psychiatric Division, Faculty of Medicine and Surgery, University of Parma, Strada del Quartiere 2, I-43100 Parma, Italy

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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

* Corresponding author at: Laboratory of Pharmaco-Toxicological Analysis, Department of Pharmaceutical Sciences, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy. Tel.: +39 051 2099739; fax: +39 051 2099740.
E-mail address: mariaaugusta.raggi@unibo.it (M.A. Raggi).

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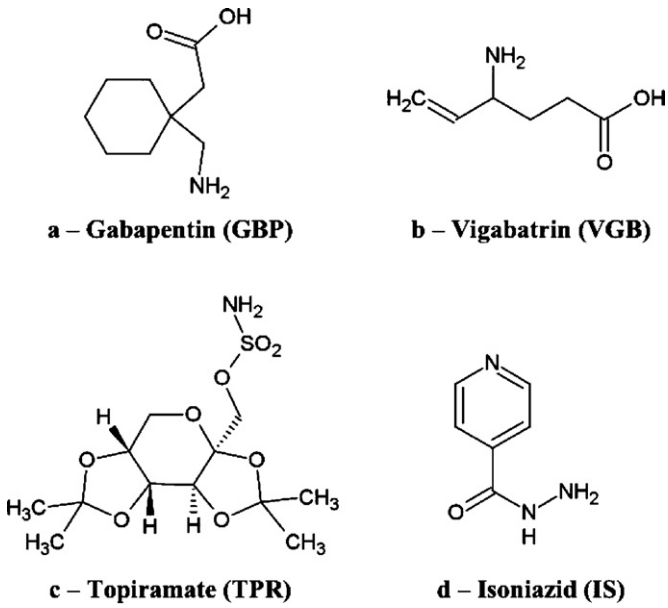

Fig. 1. Chemical structures of (a) gabapentin (GBP), (b) vigabatrin (VGB), (c) topiramate (TPR) and (d) the internal standard isoniazid (IS).

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.

The solid-phase extraction procedure for TPR was carried out on Varian BondElut Plexa cartridges (30 mg, 1 mL), conditioned with 500 μL of methanol and equilibrated with 500 μL of ultrapure water. To 200 μL of plasma, 200 μL of 0.001 N HCl and 20 μL of IS standard solution were added and the resulting mixture loaded onto a conditioned cartridge. The cartridge was then washed with 500 μL of water–methanol (95:5, v/v). The analytes were then eluted with 500 μL of methanol. The eluate was brought to dryness and re-dissolved with 200 μL of ultrapure water; 100 μL of this solution were subjected to the derivatisation procedure.

2.4. Derivatisation

To 100 μL of solution coming from the plasma sample pretreatment (or to 100 μL of standard solution), 200 μL of a 50 mM, pH 10.5 carbonate buffer and 200 μL of 2 mg mL^{-1} DC solution were added. The mixture was vortexed and then kept at 50 °C for 10 min. The resulting solution was injected into the HPLC system.

2.5. Method validation

2.5.1. Calibration curves

Aliquots of 50 μL of analyte standard solutions (prepared daily) at seven different concentrations containing the IS at a constant concentration were added to 500 or 200 μL of blank plasma. The resulting mixture was subjected to the previously described SPE procedure and injected into the HPLC. The procedure was carried out in triplicate for each concentration. The analyte/IS peak area ratios (pure numbers) obtained were plotted against the corresponding concentrations of the analytes (expressed as ng mL^{-1}) and the calibration curves set up by means of the least-square method. The values of limit of quantification (LOQ) and limit of detection (LOD) were calculated according to USP [22] and "Crystal City" [23] guidelines as the analyte concentrations which give rise to peaks whose heights are 10 and 3 times the baseline noise, respectively.

2.5.2. Extraction yield (absolute recovery)

The procedure was the same as that described under Section 2.5.1, except the points were at three different concentrations, corresponding to the upper limit, lower limit and middle point of each calibration curve. The analyte/IS peak area ratios were compared to those obtained by injecting standard solutions at the same theoretical concentrations and the extraction yield values were calculated.

2.5.3. Precision

The assays described under Section 2.5.2 were repeated six times within the same day to obtain repeatability (intraday precision) and six times over six different days to obtain intermediate precision (interday precision) [23], both expressed as RSD% values.

2.5.4. Selectivity

Blank plasma samples from six different volunteers were subjected to the SPE procedure and injected into the HPLC; the resulting chromatograms were checked for possible interference from endogenous compounds. The acceptance criterion was, that no interference peak is to be higher than an analyte peak corresponding to its LOD. Furthermore, standard solutions of several different drugs active on the central nervous system were injected at concentrations higher than the respective therapeutic levels; if the resulting chromatograms contained any potentially interfering peak, the compounds were then subjected to the SPE and injected to ascertain if they could be extracted.

2.5.5. Accuracy

Accuracy was evaluated by means of recovery assays. The assays described under Section 2.5.2 were carried out adding standard solutions of the analytes and the IS to real plasma samples taken from depressed patients subjected to therapy with GBP, VGB and/or TPR. The assays were repeated three times during the same day to obtain mean recovery and SD data.

3. Results and discussion

3.1. Derivatisation procedure

Since the three analytes (GBP, VGB and TPR) do not possess any significant chromophore in their chemical structures, fluorescent derivatisation was chosen in order to obtain the sensitivity needed for the analysis in human plasma. Among the different possible fluorescent probes, DC is particularly attractive since it does not show any native fluorescence itself; only the reaction product emits light when excited at a wavelength of 300 nm. This is a great advantage, because the excess reagent is often a possible source of interference.

The analyte: DC molar ratio, reaction time and temperature were studied in order to find the optimal conditions. Preliminary assays showed that a 1:2 analyte:DC molar ratio is sufficient to achieve complete analyte derivatisation. Application to biological samples is not expected to require changes, since the samples are subjected to a pre-treatment by SPE, eliminating most compounds that could react with DC. The effect of temperature on reaction rates was studied in the 25–60 °C range. As expected, increasing the temperature shortens reaction times: for example, the time required by the reaction to reach completeness is 1 h at 30 °C, while it is 10 min at 50 °C, without significant differences among the analytes. These conditions were thus chosen for the subsequent assays, since it was also observed that heating over 50 °C causes significant analyte losses.

3.2. Chromatographic conditions

The analyte-DC derivatives of GBP and VGB are much more retained than that of TPR on a normal C8 column. For this reason, it was chosen to use a Hydro-RP sorbent that is similar to a C18, but it is modified with patented functional groups that enhance the retention of hydrophilic compounds and increase its selectivity toward lipophilic compounds. Moreover, having 5-μm silica particles, it grants higher efficiency when compared to the previously published method that uses pre-column derivatisation with DC [21] and a column with 10-μm particles. This choice proved to be suitable, since all analyte derivatives could be detected within reasonable times (17 min), using a slightly acidic mobile phase composed of acetonitrile and phosphate buffer (40:60, v/v). In order to further reduce chromatographic times, the acetonitrile percentage was increased to 45% and a flow rate gradient (from 1.0 to 2.5 mL min^{-1}) was programmed, obtaining the complete resolution of the analytes in less than 12 min. Isoniazid was selected as the IS.

The chromatogram of a standard solution (after the derivatisation procedure) containing the analytes and the IS is shown in Fig. 2. As can be seen, the peaks are neat, symmetric and well separated.

3.3. Analysis of standard solutions

Seven-point calibration curves were set up over different concentration ranges: 1–150 μg mL^{-1} for GBP, 5–750 μg mL^{-1} for VGB and 1–50 μg mL^{-1} for TPR. Good linearity ($r^2 > 0.9997$) was obtained, with limits of quantitation (LOQ) between 1 and 5 μg mL^{-1} and limits of detection (LOD) between 0.3 and 1.7 μg mL^{-1}.

Precision was evaluated at three concentrations and RSD values were always lower than 5.0% for repeatability (intraday precision)

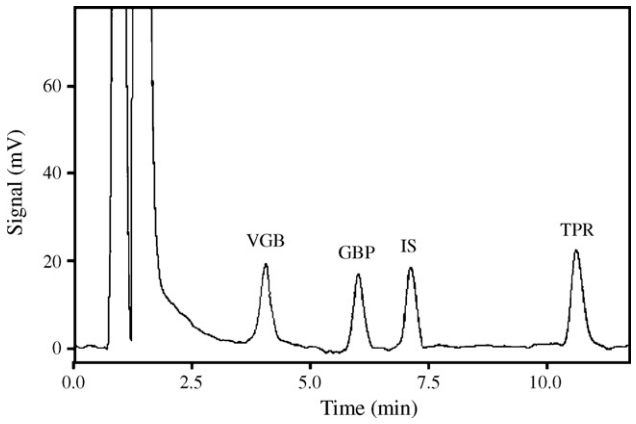

Fig. 2. Chromatogram of a standard solution containing 5 μg mL^{-1} of GBP, 30 μg mL^{-1} of VGB, 10 μg mL^{-1} of TPR and 20 μg mL^{-1} of the IS.

and lower than 5.5% for intermediate precision (interday precision).

3.4. Development of the solid-phase extraction procedure

SPE was chosen for the biological sample pre-treatment since it can confer high selectivity to the method, while granting high extraction yields; moreover, it is fast, feasible and uses small amounts of plasma sample. Different kinds of sorbents were tried, such as hydrophilic–lipophilic balance (HLB), cyanopropyl (CN), C2, C8, hydrophobic core–lipophilic surface (Plexa) and mixed-mode reversed-phase strong cation exchange (MCX). Due to the very different chemical-physical characteristics of the analytes, no single sorbent was found suitable for all of them. However, MCX cartridges proved to have sufficient affinity towards GBP and VGB, probably due to the two different mechanisms of retention: lipophilicity and cation exchange. On the other hand, the structure of TPR is not suitable for cation exchange, however it is well retained on the Plexa sorbent.

Regarding GBP and VGB, the washing steps initially consisted of 2 mL of 0.1 N HCl and 50 μL of methanol. However, strong interference was observed on VGB. Adding another washing step with methanol–phosphate buffer (20:80, v/v) greatly improved the selectivity. Elution is carried out with 2 mL of a basic water/methanol mixture, needed to quantitatively extract the IS.

Regarding TPR, the Plexa sorbent was selected because it works well with small volumes of solvents and this leads to very fast and feasible procedures. A simple washing step with 500 μL of water (containing 5% of methanol) eliminated most interference, while elution is performed with 500 μL of methanol. The sample is loaded after making it slightly acidic, in order to suppress analyte dissociation.

The chromatogram of blank plasma samples spiked with known amounts of VGB, GBP and the IS, or of TPR and the IS, are shown in Fig. 3a and b, respectively. In the blank plasma samples, no interference can be detected near the chromatographic peaks of the analytes; furthermore, peak shapes and resolution are good.

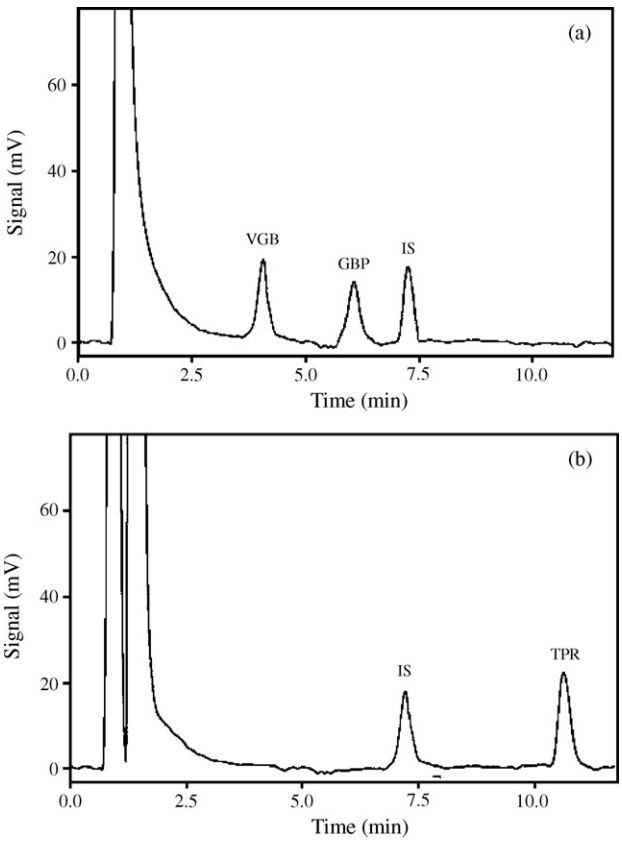

Fig. 3. Chromatograms of (a) a blank plasma sample from a healthy volunteer spiked with 1 μg mL^{-1} of GBP, 6 μg mL^{-1} of VGB and 4 μg mL^{-1} of the IS; (b) a blank plasma sample spiked with 10 μg mL^{-1} of TPR and 20 μg mL^{-1} of the IS.

3.5. Method validation

Satisfactory linearity ($r^2 > 0.9992$) was obtained over the chosen concentration ranges, which include the commonly accepted therapeutic ranges: 12–20 μg mL^{-1} for GBP [24], 10–100 μg mL^{-1} for VGB [25] and 5–20 μg mL^{-1} for TPR [24]. Linearity ranges, correlation coefficients and LOD and LOQ values are reported in Table 1.

Extraction yield (absolute recovery) assays were carried out on blank plasma spiked with analyte concentrations corresponding to the lower limit, middle point and upper limit of the respective calibration curves. As one can note, mean extraction yields were good, always higher than 91% (94% for the IS). Precision results were also satisfactory: RSD values were always lower than 5.9% (4.0% for the IS) for repeatability and lower than 6.4% (4.6% for the IS) for intermediate precision. The results of extraction yield and precision assays are reported in Table 2.

Selectivity was evaluated by injecting into the HPLC standard solutions of several drugs: other antiepileptics, antipsychotics, antidepressants and sedative-hypnotics. The complete list of the tested drugs and their retention times are reported in Table 3. As can be seen, none of them causes any interference in the analysis.

Table 1
Linearity parameters for the analytes on spiked blank plasma.

Analyte	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Linearity range (μg mL^{-1})	r^2	Linearity equation ($y = ax + b$)[a]	
					a	b
GBP	0.1	0.2	0.2–50.0	0.9993	0.640	0.028
VGB	0.3	1.0	1.0–100.0	0.9993	0.121	−0.014
TPR	0.3	1.0	1.0–50.0	0.9994	0.080	0.026

[a] y is the analyte/IS peak area ratio, a pure number; x is the analyte concentration, expressed as μg mL^{-1}.

Table 2
Extraction yield and precision values on spiked blank plasma.

Analyte	Concentration added (μg mL^{-1})	Extraction yield (%)[a]	Precision (RSD%)[a]	
			Repeatability	Intermediate precision
GBP	0.2	97	5.4	5.9
	15.0	94	5.3	5.3
	30.0	98	5.0	5.1
VGB	1.0	98	5.8	6.3
	50.0	98	5.7	6.1
	100.0	98	5.5	5.8
TPR	1.0	92	3.5	4.2
	25.0	94	3.0	4.0
	50.0	96	2.8	3.9
IS	20.0	95	3.9	4.5

[a] $n = 6$.

Furthermore, six blank plasma samples were injected after SPE and none of them produced peaks from endogenous compounds, which could interfere with the determination. Therefore, the method has demonstrated to possess satisfactory selectivity.

3.6. Analysis of patient plasma samples

Having thus validated the method, it was applied to the analysis of plasma samples from some epileptic and psychiatric patients from the Department of Neurosciences (University of Parma, Italy).

Fig. 4a–c shows the chromatograms of plasma samples from three patients treated with 1200 mg day^{-1} of GBP (a), 1000 mg day^{-1} of VGB (b), and 200 mg day^{-1} of TPR (c). The analyte concentrations found in these real blood samples were 5.1 μg mL^{-1} of GBP, 21.6 μg mL^{-1} of VGB and 9.0 μg mL^{-1} of TPR, respectively. The found plasma levels were within the reported therapeutic ranges for the three drugs, with respect to the daily oral doses. Accuracy was evaluated by means of recovery assays. Standard solutions of the analytes at three different concentrations (and of the IS at a constant concentration) were added to real plasma samples from patients, containing known amounts of the analytes

Table 3
Drugs tested for selectivity assays.

Therapeutic class	Compound[a]	t_R (min)[b]
(Analytes and IS)	GBP	6.0
	VGB	4.1
	TPR	11.1
	IS	7.3
Antidepressants	Amitriptyline	13.6
	Amoxapine	n.d.
	Clomipramine	n.d.
	Fluoxetine	n.d.
	Maprotiline	n.d.
	Mirtazapine	n.d.
	Paroxetine	n.d.
	Sertraline	n.d.
	Trazodone	n.d.
	Venlafaxine	n.d.
Antipsychotics	Amisulpride	n.d.
	Chlorpromazine	n.d.
	Clotiapine	n.d.
	Clozapine	n.d.
	Haloperidol	n.d.
	Levomepromazine	9.6
	Levosulpiride	n.d.
	Promazine	n.d.
	Quetiapine	n.d.
	Risperidone	n.d.
Anxiolytics-hypnotics	Clonazepam	n.d.
	Delorazepam	n.d.
	Diazepam	n.d.
	Flurazepam	n.d.
	Lorazepam	n.d.
Antiepileptics	Carbamazepine	n.d.
	Lamotrigine	n.d.
	Levetiracetam	n.d.
	Oxcarbazepine	n.d.

[a] All compounds were tested at a concentration of 5 μg mL^{-1}.
[b] n.d. = not detected within a 15-min chromatographic run.

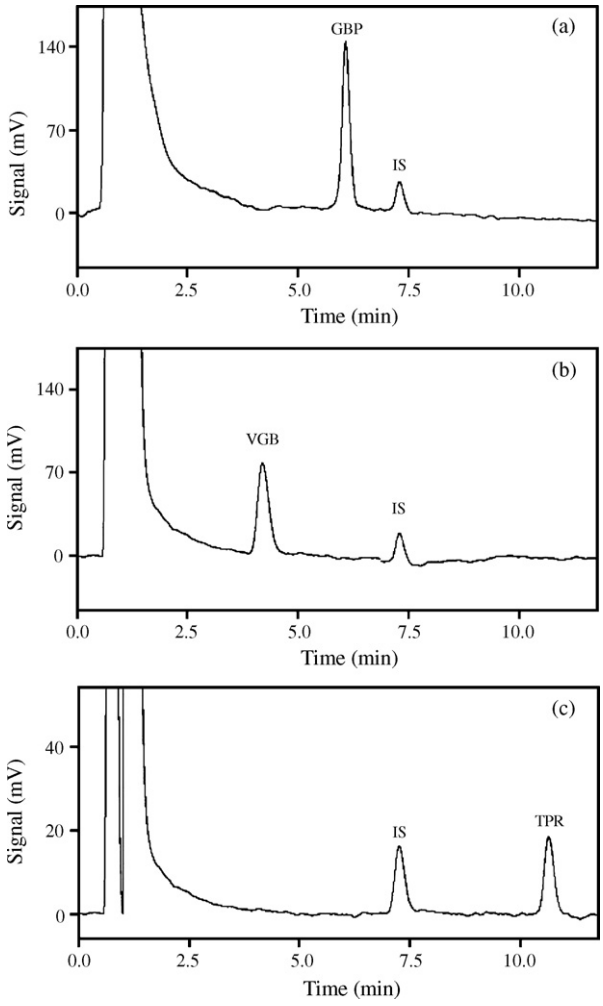

Fig. 4. Chromatograms of plasma samples from patients subjected to treatment with: (a) 1200 mg day^{-1} of GBP, (b) 1000 mg day^{-1} of VGB and (c) 200 mg day^{-1} of TPR.

Table 4
Accuracy assay results.

Analyte	Concentration added (μg/mL)	Recovery (%)	SD[a]
GBP	0.2	92	5.5
	5.0	93	4.1
	15.0	94	3.9
VGB	1.0	96	5.1
	25.0	95	4.4
	50.0	95	4.0
TPR	1.0	96	3.2
	10.0	94	3.7
	20.0	95	4.0

[a] $n = 3$.

(i.e., samples which had already been analysed). Then recovery of the added analytes was calculated. The results of the accuracy assays are reported in Table 4: mean recovery values were always higher than 91% (SD < 5.6). Thus, method accuracy is very satisfactory.

4. Conclusion

An original HPLC method with fluorescence detection has been developed for the analysis of GBP, VGB and TPR in human plasma for TDM purposes. The SPE procedures implemented for the sample pre-treatment allowed obtaining good extraction yields (>91%) for all analytes, as well as satisfactory matrix purification from endogenous interference.

When compared to existing methods, the proposed method has the evident advantage of allowing the simultaneous determination of all the three drugs: GBP, VGB and TPR. Furthermore, it does not require very expensive instrumentations (such as MS/MS detectors) [18,19], which are not always available in clinical analysis laboratories. Other methods use procedures such as protein precipitation, which produce less clean extracts and lower accuracy [20], or have lower precision and sensitivity [10,16,17].

With respect to the previous method, which uses DC as a derivatising agent [21] and only analyses GBP and VGB, this derivatisation procedure is faster (10-min reaction with DC instead of 20-min), as well the chromatographic run (12 min for three analytes instead of 14 min for two analytes). The overall method has a wider linearity (0.2–50 μg/mL for GBP and 1–100 μg/mL for VGB, instead of 5–40 μg/mL for both drugs) and the SPE procedure is more efficient then the protein precipitation.

Therefore, the proposed method has significant advantages with respect to those available in the literature; it has been successfully applied to the analysis of GBP, VGB and TPR in plasma samples from some psychotic and epileptic patients, with good accuracy and selectivity. Thus, the method seems to be useful for the TDM of patients undergoing monotherapy or polypharmacy with these drugs.

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