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Determination of topiramate in human plasma by capillary electrophoresis with indirect UV detection

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ABSTRACT

A rapid capillary zone electrophoresis method with indirect UV detection for the determination of topiramate in human plasma was developed and validated. The analyses were carried out with a background electrolyte composed of 10 mM sulfamethoxazole as chromophore in phosphate buffer (25 mM, pH 12.0); gabapentin was selected as the internal standard. Application of a voltage of +15 kV led to an analysis time shorter than 5 min; indirect UV detection was operated at 256 nm. Isolation of topiramate from plasma was accomplished by a carefully implemented solid-phase extraction procedure on C18 cartridges. The method provided a linear response over the concentration range of 2–60 μg of topiramate per mL of plasma. The limit of detection (LOD) was 0.8 μg mL^{-1} and the limit of quantitation (LOQ) was 2.0 μg mL^{-1}. Precision, expressed as relative standard deviation, was always lower than 7.3%, extraction yields were always greater than 92%. The results obtained analysing plasma samples from epileptic patients undergoing therapy with topiramate were satisfactory in terms of precision and selectivity.

1. Introduction

Topiramate (2,3:4,5-bis-O-(1-methylethylidene)-D-fructopyranose sulfamate, TPR) is an antiepileptic drug with a monosaccharide structure, chemically unrelated to other classes of antiepileptics. It is currently used as an adjunctive agent for the control of partial and generalised seizures in both adults and children [1], but is also approved and frequently used for the prevention of migraine [2]. Moreover, there is growing evidence in the literature [3] to support the use of TPR in both the treatment of alcohol withdrawal and the prevention of alcohol relapse [4]. However, none of these indications have yet been approved by the FDA.

The exact mechanism of action is still not completely known; however, TPR influences multiple systems: it probably blocks voltage-dependent sodium channels, enhances GABAergic transmission, acts as antagonist at AMPA/kainate glutamatergic receptors and inhibits carbonic anhydrase [5].

TPR is administered as Topamax® tablets (sprinkle capsules and generic formulations also exist) at daily doses usually ranging from 200 to 400 mg, however up to 1600 mg day^{-1} can be applied when needed. The drug is rapidly absorbed (within 2 h) and bioavailability is about 80% [6]. Metabolism is scarce: no active metabolite is known and the parent drug is almost completely excreted unmodified in the urine. Therapeutic plasma levels are generally considered to be in the 5–20 μg mL^{-1} range [7]. Side effects are usually dose-dependent, thus more frequent and severe with daily doses above 400 mg; vertigo, drowsiness, ataxia, headache and difficulty in thinking and concentrating are the most frequent ones [8]. Since TPR also inhibits carbonic anhydrase, cases of nephrolithiasis and metabolic acidosis have been observed, especially in cases of polypharmacy with diuretics, such as acetazolamide [9].

As with several other antiepileptics, constant monitoring of TPR plasma levels is recommended to obtain optimal therapeutic results [10]. However, since the drug does not possess any significant chromophores (see structure in Fig. 1), its quantitation by conventional spectroscopic means is not straightforward.

Only one paper [11] reports a method, which makes use of HPLC and UV detection for the analysis of TPR in human serum, but it requires derivatisation using the fluorescent reagent 9-fluorenylmethyl chloroformate in order to make the molecule detectable. Another method by the same authors [12] adopts another fluorescent labelling agent (4-chloro-7-nitrobenzofurazan) and fluorescence detection, which enables the determination of TPR at concentrations down to 0.010 μg mL^{-1}.

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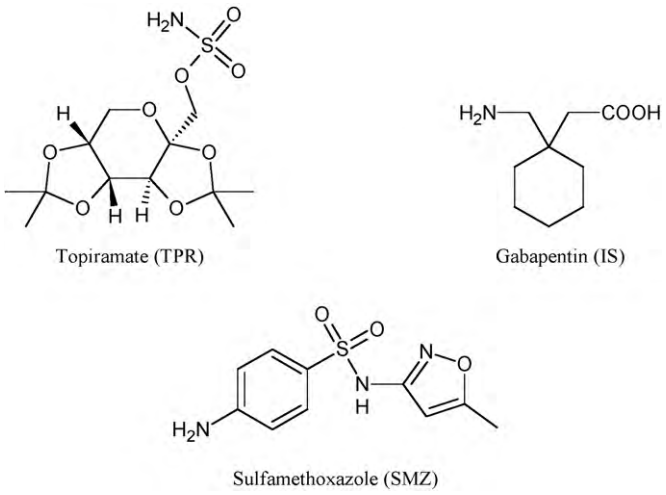

Fig. 1. Chemical structures of topiramate (TPR), gabapentin (IS) and sulfamethoxazole (SMZ).

Other papers avoid the derivatisation step and use mass spectrometric detection [13–15].

An alternative to chromatography for ionisable molecules is capillary electrophoresis (CE). TPR is a slightly acidic compound, and can thus potentially be analysed by CE at high pH. In order to avoid derivatisation when using a UV absorbance detector, we have applied indirect UV detection. Although the sensitivity of this detection method is not outstanding, it can be sufficient since the therapeutic concentration of TPR in biological fluids ranges in the tens of $\mu g\,mL^{-1}$. It was thus the aim of this paper to develop a fast, feasible and validated method for the quantitative analysis of TPR in human plasma based on capillary zone electrophoresis (CZE). No method currently exists for the analysis of TPR in biological fluids by CZE; only its impurities (sulphate and sulphamate) have been analysed in the pure compound with this technique [16].

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were of analytical grade or better. Topiramate, gabapentin used as the internal standard (IS), sulfamethoxazole, potassium dihydrogen phosphate, and methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dibasic sodium phosphate, sodium chloride and potassium chloride were from Carlo Erba (Milan, Italy). Ultrapure water (18.2 MΩ cm), obtained by means of a Millipore (Billerica, MA – USA) Milli-Q apparatus, was used for the preparation of all solutions.

2.2. Instrumentation and electrophoretic conditions

All CZE experiments were carried out using a ^{3D}CE apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a photodiode array (PDA) detector. Uncoated fused silica capillaries (75 μm I.D., 375 μm O.D., 48.5 cm total length, 40.0 cm effective length) from Composite Metal Services (Ilkley, UK) were used. The indirect UV determination of TPR was performed using 10 mM sulfamethoxazole as the chromophore in a phosphate buffer (25 mM, pH 12.0) as the background electrolyte (BGE). It was prepared as follows: 88.7 mg of potassium dihydrogen phosphate were dissolved in about 40 mL of water; this solution was then adjusted to pH 12.0 with 1 M NaOH and made up to 50 mL in a volumetric flask. Finally, 12.7 mg of sulfamethoxazole were weighed and dissolved in 5 mL of the phosphate buffer. The BGE was filtered through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs-020-25) prior to use.

Injection was carried out by pressure at the anodic end of the capillary at 50 mbar for 5 s. The applied voltage was set at +15 kV, and the capillary was thermostatted at 25.0 °C. The detection wavelength was 256 nm.

Before use, the new capillary was conditioned by rinsing with 1 M sodium hydroxide, water, and then with the BGE for 10 min each. After each run the capillary was rinsed with BGE for 2 min. For storage overnight, the capillary was washed with water and additionally with 1 M sodium hydroxide, and again with water (rinsing time was 5 min each).

2.3. Preparation of standard solutions

The stock solutions ($2.00\,mg\,mL^{-1}$) of TPR and the IS were prepared by dissolving a suitable amount of pure TPR substance in 10 mL of methanol. Standard solutions of the individual compounds were prepared by diluting suitable amounts of each stock solution with water. The standard working solutions were prepared every day, while the stock solutions of the analyte and the IS in methanol were stable for at least three months when stored at −20 °C, as assessed by CE analysis.

2.4. Sample collection and preparation

Blood samples were collected from patients of the Division of Psychiatry, University of Parma (Italy) under polypharmacy with antipsychotics and TPR, and the blood put into vials containing EDTA as the anticoagulant. The blood was immediately centrifuged for 20 min at 3000 rpm and the supernatant plasma was frozen and maintained at −20 °C until analysis. The same procedure was used to obtain plasma from the blood of healthy volunteers ("blank" plasma).

Sample pre-treatment was carried out by solid-phase extraction (SPE) using reversed-phase Varian (Harbor City, CA, USA) BondElut C18 cartridges (100 mg, 1 mL).

Before use, the cartridges were conditioned by flushing them 5 times with 1 mL of methanol and then equilibrated with 1 mL of 100 mM KH_2PO_4 5 times.

Plasma aliquots (500 μL) were spiked with the IS (100 μL) and mixed with 1 mL of 100 mM KH_2PO_4. The solution obtained was loaded onto the previously conditioned cartridge. The washing procedure consisted of 1 mL of 100 mM KH_2PO_4, followed by 1 mL of water.

Elution was carried out with 2 mL of methanol. The eluate was then dried by means of a rotary evaporator and reconstituted with 100 μL of ultrapure water.

2.5. Method validation

All assays were carried out according to USP XXIX [17] guidelines.

2.5.1. Linearity

A calibration curve was obtained by spiking blank plasma with seven different concentrations of TPR and constant concentration of the IS, followed by the SPE procedure and injection. The calibration graph was calculated from the measured TPR/IS area ratio values as function of the analyte concentrations added to blank plasma by means of the least square method.

2.5.2. Precision

The blank plasma was spiked with TPR at three different concentrations (with constant IS concentration) to give final TPR concentrations corresponding to the lower limit, a middle value