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Short communication

A direct HPLC method for the resolution and quantitation of the R-(−)- and S-(+)-enantiomers of vigabatrin (γ-vinyl-GABA) in pharmaceutical dosage forms using teicoplanin aglycone chiral stationary phase

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A direct chiral high-performance liquid chromatography (HPLC) method was developed and validated for the resolution and quantification of antiepileptic drug enantiomers, R-(−)- and S-(+)-vigabatrin (gamma-vinyl-gamma-aminobutyric acid) in pharmaceutical products. The separation was optimized on a macrocyclic glycopeptide antibiotic chiral stationary phase (CSP) based on teicoplanin aglycone, chirobiotic (TAG), using a mobile phase system containing ethanol–water (80:20, v/v), at a flow rate of 0.4 ml/min and UV detection set at 210 nm. The stability of vigabatrin enantiomers under different degrees of temperature was also studied. The enantiomers of vigabatrin were separated from each other. The calibration curves were linear over a range of 100–1600 μg/ml (r = 0.999) for both enantiomers. The overall recoveries of R-(−)- and S-(+)-vigabatrin enantiomers from pharmaceutical products were in the range of 98.3–99.8% with %RSD ranged from 0.48 to 0.52%. The limit of quantification (LOQ) and limit of detection (LOD) for each enantiomer were 100 and 25 μg/ml, respectively. No interferences were found from commonly co-formulated excipients.

1. Introduction

Increasing demands for the separation of chiral compounds, and production of enantiomerically pure compounds have led to enantioselective separation becoming one of the most important analytical task [1]. Over the past few years, it has been demonstrated that the chiral stationary phases (CSPs) based on macrocyclic antibiotics (teicoplanin, teicoplanin aglycone, vancomycin, ristotecin A, etc.) are extremely useful for enantiometric separations of racemic compounds and appear particularly suitable for preparative chromatography [2–4]. One of the recent selectors is teicoplanin. The aglycone-derivative of teicoplanin appears to be especially suited for the separation of underivatized amino acids [5]. Teicoplanin aglycone TAG differs from teicoplanin by the lack of the sugar chains.

Vigabatrin (γ-vinyl-γ-aminobutyric acid, γ-vinyl-GABA) is one of the newer generation of antiepileptic drugs. It is a structural analogue of γ-aminobutyric acid (GABA) (Fig. 1). It acts by irreversibly inhibiting the enzyme GABA transaminase in brain, increasing GABA concentrations and reducing seizure activity [6]. Nowadays, vigabatrin is regarded by many authorities as a drug of choice in infants with West syndrome (infantile spasms), particularly in cases associated with tuberous sclerosis [7,8]. Vigabatrin is supplied as a racemic mixture of the enantiomers, but only the (S)-(+)-enantiomer is pharmacologically active [9]. Therefore it is useful to provide chiral separation and enantiometric analysis methods of this drug.

In the past decade, several analytical methods, such as high-performance liquid chromatography (HPLC) method [10–12] and capillary electrophoresis (CE) method [13,14] for the determination of vigabatrin have been developed. However, only few methods have been described to date for the chiral separation of vigabatrin enantiomers. Haegele et al. [15] reported a gas chromatography–mass spectrometry (GC–MS) method for the determination of vigabatrin enantiomers. Schramm et al. [16] developed a gas–liquid chromatography (GLC) method to detect the content of vigabatrin enantiomers in plasma or serum. Although GC methods are relatively sensitive, they require complex sample preparation involving double derivatization of the drugs to improve the volatility and avoid column interactions. An HPLC method with pre-column derivatization has also been presented for the analysis of vigabatrin enantiomers [17,18]. But the method of derivatization may often be time-consuming, frequently requires strict control to temperature and rigorous sample clean up or undergo cross-reaction during the analysis. The direct resolution of racemic vigabatrin by HPLC without derivatization is very rare. Recently, Lee et al. attempted to resolve racemic vigabatrin and its analogue γ-amino acids on liquid chromatographic chiral stationary phases

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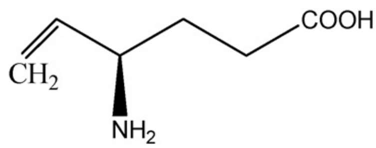
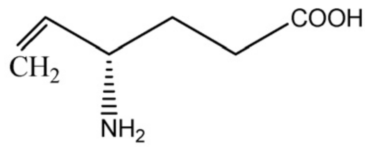


(A)



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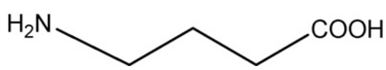

(C)

Fig. 1. The chemical structure of (A) S-(+)-vigabatrin, (B) R-(−)-vigabatrin, and (C) GABA.

(CSPS) based on (+)-(18-crwon-6)-2,3,11,12-tetracarboxylic acid [19], but this method was only dealing with resolution of vigabatrin enantiomers and its analogue and was not applied for the analysis of the drug in its pharmaceutical dosage forms.

The aim of this work was to develop and validate a simple stereoselective HPLC method with UV detection based on the use of a teicoplanin aglycone macrocyclic antibiotic CSP for the direct enantioselective analysis of vigabatrin in pharmaceutical formulations. With the present broad range of available CSPs and advances in column technology, the present enantioselective HPLC can be considered as the method of choice for the quantitation of racemic vigabatrin without previous derivatization.

2. Experimental

2.1. Chemical and reagents

(±)-vigabatrin, S-(+)-vigabatrin and R-(−)-vigabatrin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ethanol as HPLC-grade was purchased from BDH Chemicals (Poole, UK). Water was deionized and doubly distilled using a cartridge system (Picotech water system, RTP, NC, USA). Sabril tablets (containing 500 mg of vigabatrin as racemate/tablet) were purchased from the local market.

2.2. Instrumentation and chromatographic conditions

The resolution of the enantiomers was performed on a Waters Breeze system consisting of a 1525 Binary HPLC pump, 717 plus autosampler, 2487 Dual channel absorbance detector and In-line degasser AF (Milford, MA, USA). The column used for the analytical separation was the macrolide-type antibiotic teicoplanin aglycone known as chirobiotic TAG (15 cm × 4.6 mm i.d., 5 μm particle size) purchased from Advanced Separation Technologies (Whippany, NJ, USA).

The mobile phase consisted of ethanol:water (80:20, v/v) and was filtered through a Millipore membrane filter (0.2 μm) from Nihon, Millipore (Yonezawa, Japan). The flow rate was 0.4 ml/min, the sample injection volume was 20 μL.

The appropriate wavelength for the detection of the drug was determined by wavelength scanning over the range of 200–400 nm with a Shimadzu UV-double beam spectrometer and the chromatograms were monitored by UV detection at a wavelength of 210 nm.

2.3. Preparation of standard stock solutions

Stock solutions of S-(+)-vigabatrin, R-(−)-vigabatrin (2.0 mg/ml) were prepared by dissolving the appropriate amount of substances in water. A seven-point non-zero calibration standard curve, ranging from 100 to 1600 μg/ml, was prepared.

2.4. Preparation of standard solutions of tablets

An accurately weighed amount equivalent to 50 mg vigabatrin was transferred into 100 ml volumetric flask with the aid of 50 ml of water and stirred for 10 min. The extract was filtered and complete to volume with water. Accurately measured aliquots of the supernatant were transferred to 5 ml volumetric flasks diluted to 5 ml with water to give final concentration of 250, 750 and 1600 μg/ml of vigabatrin.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

In order to get optimum resolution and selectivity for the two enantiomers from pharmaceutical preparations, various macrocyclic antibiotic CSPs and various experiments were conducted. The chiral separation was optimized using isocratic conditions as these offer more rapid analysis attributable to the presence of column re-equilibration steps.

The separation of vigabatrin enantiomers was first attempted using vancomycin CSP, teicoplanin CSP and ristotecin. However, despite the use of a range of different possible mobile phase compositions, no separation was achieved for vigabatrin enantiomers on any of the three columns (data not shown). In order to improve the resolution of vigabatrin enantiomers, teicoplanin aglycone chiral stationary phase (TAG CSP) was used.

Several mobile phase compositions were tested on teicoplanin TAG CSP. With the mobile phase consisted of methanol or ethanol, no enantioseparation was observed in the absence of water. While with methanol in the presence of water, separation was poor, with ethanol, the enantioresolution increased with increasing water concentration in the mobile phase. An increase of water concentration in ethanol in the mobile phase to about 15%, partial enantioseparation of the studied enantiomers were obtained (Rs = 1.1). Increasing the concentration of water with ethanol to about 20%, a significant improvement in both the resolution and sensitivity was achieved (Rs = 2.91). The increase in resolution with increasing water content was due to enhanced hydrophobic interactions in the water-rich mobile phase, while the increase in the resolution with increasing ethanol content was due to the decreased solubility of polar vigabatrin in ethanol-rich mobile phase [20].

It was also found that no significant influence of the pH in the range of 5–7 was observed on retention or separation. This can be easily explained by the fact that the vigabatrin molecule is present in its zwitterionic form at this pH range (carboxylic moiety pKa ∼4.0, amine moiety 8.6) and the function groups of the tiecoplanin-aglycone molecule involved in the chiral discrimination also have their pKa values (acidic group ∼4.0, basic group ∼9.2 [5]) far from this pH change.

From this study, the optimized conditions of ethanol–water (80:20, v/v) (Table 1) were established as the final mobile phase conditions with TAG CSP at flow rate 0.4 ml/min. This simple mobile phase is advantageous particularly if method is transferred to