Determination of Optical Impurity of Pregabalin by HPLC with Pre-Column Chiral Derivatization

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Abstract

A new method for determining the optical impurity of Pregabalin is developed. The method is based on Pregabalin, and its isomers can be derivatized with Nᵃ-5-fluoro-2,4-dinitrophenyl-5-L-alanine amide. These derivated compounds can be separated by an ordinary chromatography column (Inertsil ODS-2.5 µm, 250 mm × 4.6 mm i.d.). Phosphoric acid buffer and acetonitrile (55:45, v/v) are used as mobile phase and 1.0 mL/min flow rate at room temperature. The detective wavelength is fixed at 340 nm. The results indicate that the limit of detection of R model optical impurity 1.1 × 10–8 g/mL (signal-to-noise = 3), accuracy, and repeatability is satisfied. Therefore, the method can be used for the quality control of Pregabalin.

Introduction

Pregabalin was authorized by the FDA and launched in June 2005, exhibiting a very good effect on neuropathic pain and epilepsy (1). It is a γ-amino acid containing one chiral carbon. The S model is applied for medicine (see Figure 1). Enantiomers of chiral drugs often have different pharmacological actions or toxicities, the metabolism in the body is often different (2–4). Therefore, only the single isomer is applied for clinical use. So, we should separate and detect the enantiomer of impurity. For Pregabalin, only the S model is bioactive, so the isomer of the R model was controlled as optical impurity.

Currently, HPLC applying to the analysis of Pregabalin by 1-fluoro-2,4-dinitrobenzene (DNFB) and *o*-phtaldialdehyde derivatization have been reported (5–6), but this method cannot separate enantiomers of Pregabalin. The separation and determination method of the optical isomers have not been reported. Optical isomers of amino acid are generally separated by chiral bonded stationary phase (CSP) column in high-performance liquid chromatography (HPLC) (7,8); some chiral columns were experimented with, such as AD, OD, AD-H, Ceramospher chiral RU-1, Ceramospher chiral RU-2, and CROWNPAK CR (+), but none could separate enantiomers of Pregabalin. It may be that CSP column usually used for separation α and β-amino acid, γ-amino acid may be difficult for them. In addition, Pregabalin has nearly no UV absorption, so optical isomers of amino acid analysis are usually used in the pre-column derivatization method (9,10). *O*-Phthaldiadehyde (OPA) (11), 9-fluorenylmethyl chloroformate (FOMC-Cl) (12), and DNFB (13) are usually used as derivatization reagents. We detected optical isomers successfully by using Nᵃ-5-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDNPAA) as derivatization reagent. The method can be used for determining the optical impurity of Pregabalin and preparations containing Pregabalin.

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Materials and Methods

Materials

Nᵃ-5-Fluoro-2,4-dinitrophenyl-5-L-alanine amide was from Sigma (St. Louis, MO), and acetonitrile (HPLC grade) was from Burdick & Jackson (Muskegon, MI). All other reagents were of analytical grade. Three batches of Pregabalin samples (050301, 050302, 050303, content ≥ 99.5%), Pregabalin capsule (Pfizer, New York, NY), and Pregabalin racemate (050201, Content ≥ 99.5%) were used. All samples were provided by Chongqing Pharmaceutical Research Institute CO., LTD (Chongqing, P.R. China).

Sample preparation

Eighty milligrams of Pregabalin sample were accurately weighed into 10-mL calibrated flasks and dissolved in 2 mL of 1

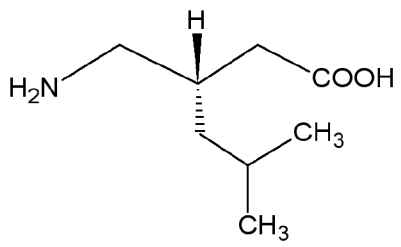

Figure 1. Molecular structure of Pregabalin.

mol/L hydrochloric acid. The pH was adjusted to 7.0 with sodium hydroxide solution, then completed to 10 mL by the addition of distilled water. Fifty microliters of the solution were measured into a 2-mL vessel, 200 µL of the FDNPAA solution (100.0 mg FDNPAA was dissolved by acetone into a 10-mL calibrated flask and stored in the refrigerator), and 20 µL 1 mol $NaHCO_3$ were added. The solution was closed and heated for 1 h at 40°C, then 10 µL 2 mol/L hydrochloric acid was added, and the solution was dried in vacuum desiccation oven (Shanghai Yi Chuan instrument CO., LTD, Shanghai, P.R. China) with P_2O_5/KOH desiccative. The residue was dissolved in 4 mL dimethyl sulfoxide (DMSO) (equivalent to100.0 µg/mL Pregabalin sample).

HPLC analysis

The sample solution was filtered through a membrane filter, and an aliquot (20 µL) of the sample was injected for HPLC analysis. The HPLC system consisted of an Agilent 1100 chromatograph (Agilent Technologies, Palo Alto, CA) with a pump (G1312A), an auto-injector (G1313A), and a diode-array detector (G1315B). Data analysis and processing were done by Agilent ChemStation software. Analyses were carried out using a Inertsil ODS-2 column (5 µm, 250 mm × 4.6 mm) (GL Sciences, Inc., Tokyo, Japan), mobile phase of phosphate buffer (7.0 mL triethylamine dissolved in 900 mL of distilled water and adjusted to pH 3.0 with phosphoric acid, then completed to 1000 mL with distilled water)–acetonitrile (55:45, v/v), flow rate of 1.0 mL/min at room temperature, monitored at 340 nm.

Results and Discussion

Optimization of mobile phase

With the C-18 column (Inertsil ODS-2) for HPLC mobile phase optimization, different ratios of acetonitrile–water, including 50:50, 70:30, and 30:70 (v/v) were examined, but a sharp peak was not observed. Adjusting the pH values of water to 3.0, 4.0, 7.0, the best chromatogram was obtained at pH 3.0, and when the organic content was increased, the retention time was shortened. Finally, the mobile phase ratio was phosphate buffer–acetonitrile (55:45, v/v). Resolution of S and R model is 4.03 at this condition, with a run time of 8.3 and 10 min.

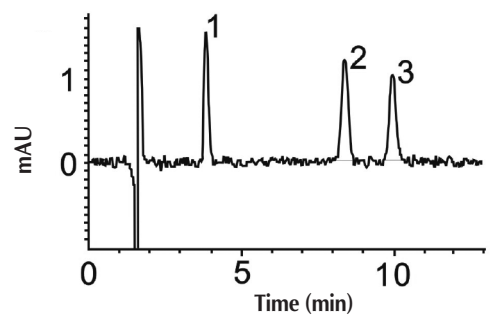

Figure 2. Chromatograms of Pregabalin racemate (0.1 µg/mL). Peaks: FDNPAA, 1; Pregabalin derivatized by FDNPAA, 2; Optical isomerism of Pregabalin derivatized by FDNPAA, 3.

Linearity of Pregabalin optical impurity at low concentration

We especially inspected the linear response of the R mode at low concentrations. Accurately weighed 160.0 mg of Pregabalin racemate (containing 80.0 mg R model impurity) was handled according to the "Sample preparation" section; 0.1, 0.2, 0.5, 1.0, 1.6, and 2.0 mL of the prepared solution were diluted in five 100-mL calibrated flasks with DMSO and 20 µL was injected for HPLC analysis, respectively. The linear equation: $Y = 139.825X + 0.0808$ (X is the concentration of the R model, Y is the area of the peak, and the correlation coefficient: $r = 0.99998$). This indicates that the R model impurity had good correlation in linearity of 5.01×10^{-6} g/mL – 1.02×10^{-4} g/mL. Method reproducibility of the R model was determined by measuring samples on one day and another day six times. Counting the intra- and interday reproducibility, the interday RSD was 0.75%, and the intraday RSD was 0.90%.

At this condition, the limit of detection of the R model impurity was 1.1×10^{-8} g/mL (signal-to-noise [S/N] = 3), and the limit of quantitation was 3.3×10^{-8} g/mL (S/N = 10). Figure 2 shows the chromatogram of a sample at 0.1 µg/mL. Using a diode-array detector (DAD) to determine the chromatographic purity of peak 1 and peak 2, the purity factors of two peaks are up to 999.95 (14), demonstrating the chromatographic peaks represent pure substances.

Stability and precision

The same sample was determined by HPLC in different times and the results shown the solution was stable for 90 min at least; the RSD is 1.32%. After six consecutive injections, the RSD is 1.08%.

Usage of FDNPPA and reaction time of derivatization

For five 50 µL Pregabalin racemate solutions, the described experiment was performed, and the mole ratio of derivatization

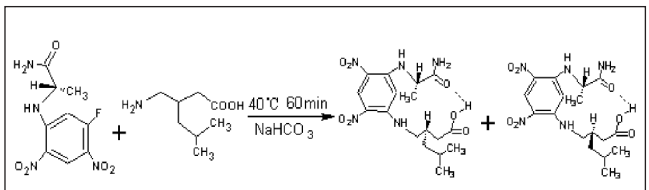

Figure 3. Mechanism for separation by C-18 column.

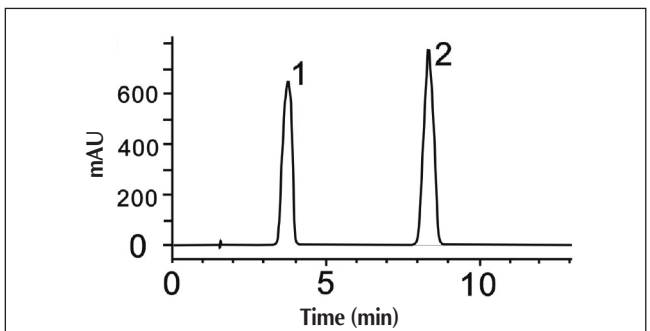

Figure 4. Chromatograms of Pregabalin (100 µg/mL). Peaks: FDNPAA, 1; Pregabalin derivated by FDNPAA, 2.

reagents and Pregabalin were 2.96:1, 3.7:1, 4.44:1, 5.18:1, and 5.92:1. The results show when the mole ratio was between 2.96:1 –5.92:1, the chromatographic areas did not change, which shows that the ratio of 3:1 for derivatization reagents and Pregablain was enough.

For 50 µL Pregabalin racemate solution, reaction times of derivatization are 40, 60, 80, 100, 120 min, respectively; the results show when time is beyond or just 60 min, the chromatographic peaks reach a stable value, so 60 min of the reaction time was ascertained.

Separation mechanism

Pregabalin has nearly no UV absorption; FDNPAA can react with amino quantitatively. The racemate sample (R and S model by equivalent content) can quantitatively react with FDNPAA rapidly, and the derivatives have UV absorption. The formed derivatives have a difference in polarity, so it can be separated with an ordinary C-18 column (Figure 3).

Determination of Pregabalin samples

Take racemates as contrast, 3 batches of Pregabalin samples (050301, 050302, 050303) and Pregabalin capsule, derived as described earlier, were determine by HPLC. Chromatographic peaks of the R model enantiomer have not been found (Figure 4). Adding certified Pregabalin into the one batch of sample, the same result has been obtained. It indicates the optical impurity is no more than 0.02% in our samples.

Conclusions

Pregabalin racemate derivated can be separated on an ordinary C18 column and can be determine by a UV detector. FDNPAA and Pregabalin can react rapidly and quantitatively. The absorption wavelength of the derivates of Pregabalin is the same as FDNPAA. The established method can be applied for determining the optical impurity of Pregabalin samples.

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