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LC method for the determination of oxcarbazepine in pharmaceutical preparations

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Abstract

A simple and accurate HPLC method for determination of oxcarbazepine (OXC) in a new tablet formulation is described. Chromatographic separation was achieved on a Diamonsil C18 column using a mobile phase consisting of acetonitrile, potassium phosphate monobasic buffer (pH 6.8) and water (36:8:56, v/v) at a flow rate of 1.0 ml/min. Absorbance was monitored at 255 nm where OXC has maximum absorption. The linear range of detection for OXC was from 9.96 to 99.6 μg/ml. The proposed method was validated for selectivity, precision, accuracy and limits of detection and quantitation, etc.

Keywords: HPLC; Oxcarbazepine; Tablet formulation

1. Introduction

Oxcarbazepine (OXC) is a novel antiepileptic drug, which was developed as a second-generation and follow-up compound to carbamazepine (CBZ). OXC has a similar therapeutic profile to CBZ but produces much less side effects on patients. Clinically it has been used to treat several types of epilepsy [1–4]. The chemical structures of OXC and CBZ are shown in Fig. 1. A new tablet formulation of OXC has been newly developed by Shenyang Pharmtech Institute of Pharmaceuticals. For the new product of OXC, it is necessary to find an analytical method to determine OXC in the tablets. Since OXC and its pharmaceutical formulations have not been listed in any of the Pharmacopoeias yet and no references describing the determination of OXC in pharmaceutical preparations have been found in publications and even the related reports are quite limited [5–10], an analytical method needed to be developed for the quality control of the product. HPLC has been widely used in the analysis of pharmaceutical compounds and formulations due to its high efficiency of separation and quantitation. The objective of this study was to develop and validate

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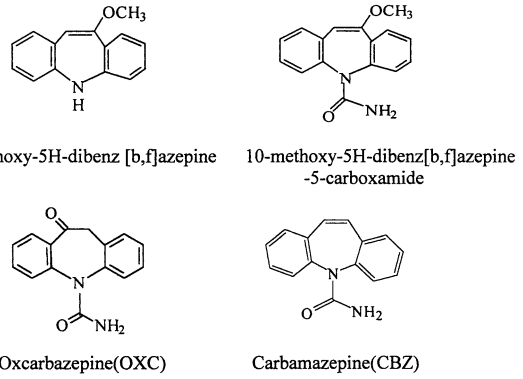

Fig. 1. Chemical structures of OXC and its related compounds.

an HPLC method for the determination of OXC in the new formulation, which can be used for the quality control of the product.

2. Experimental

2.1. Chemicals and reagents

OXC standard sample and tablets (300 mg per tablet) were provided by Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). HPLC-grade acetonitrile from Tedia Company, Inc. (USA) was used. HPLC-grade water was prepared by using a Milli-Q water purification system from Millipore (Molsheim, France). Potassium dihydrogen phosphate was from Merck (Darmstadt, Germany). All other reagents used were of analytical grade.

2.2. Instrumentation

Chromatographic separation was performed on an HP series 1100 chromatographic system equipped with G1310A Iso Pump, HP variable UV/VIS detector, G1328A Manual Injector with 20 μl loop (Agilent, USA). EChrom 98 Chromatography Workstation from Elete (Dalian, China) was used for data collecting and processing. A Diamonsil C18 column (150 × 5.0 mm, 5 μm) was from Dikma (Beijing, China). A Shimadzu UV-2201 UV/VIS double-beam spectrophotometer was used for scanning and selecting the working wavelength of detection.

2.3. Chromatographic conditions

The C18 column was used at ambient temperature. The mobile phase consisting of a mixture of acetonitrile, potassium phosphate monobasic buffer (pH 6.8) and water (36:8:56, v/v) was delivered isocratically at a flow rate of 1.0 ml/min. Prior to use, mobile phase was filtered through 0.45 μm Millipore membrane filters and degassed by sonication in an ultrasonic bath. Detector was set at 255 nm where OXC has maximum absorption. The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and samples were analyzed. The injection volume was 20 μl.

2.4. Assay of tablets

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 25 mg of OXC was transferred to a 50-ml volumetric flask. After 20 ml of the mobile phase was added into the flask, the solution was socinated for 5 min to make OXC dissolved. Then the solution was diluted to volume with the mobile phase, mixed and filtered. After discarding the first 10-ml of filtrate, 1 ml of the following filtrate was quantitatively transferred to a 10 ml volumetric flask and diluted to volume with the mobile phase. A standard solution containing 50 μg/ml of OXC was also prepared. The sample solution and standard solution were filtered through 0.45 μm Millipore membrane filters and separately injected into the chromatographic system and the chromatograms and peak areas were recorded.

3. Results and discussion

3.1. Selection of the chromatographic conditions

During our method development, top priority was given to complete separation of OXC from its

related compounds such as its synthetic precursors, intermediates, CBZ and forcedly degraded products, which completed a library of compounds used to investigate method selectivity. The starting compound and intermediate for OXC synthesis were 10-methoxy-5H-dibenz[b,f]azepine and 10-methoxy-5H-dibenz[b,f]azepine-5-carboxamide, respectively. Since the process of OXC synthesis may also produce CBZ, it is necessary for the developed method to separate OXC from CBZ and determine the existence of CBZ in OXC drug substance. The structures of 10-methoxy-5H-dibenz[b,f]azepine and 10-methoxy-5H-dibenz[b,f]azepine-5-carboxamide are shown in Fig. 1.

The most widely used solvents for HPLC include methanol, acetonitrile, water, and different kinds of buffers such as phosphate buffer, acetate buffer, etc. The mobile phase was chosen after several trials with acetonitrile, phosphate buffer and water in various proportions and at different pH values. When the proportions of acetonitrile, phosphate buffer and water were adopted separately in 20:10:70, 36:8:56 and 50:6:44, it was found that with the increase of acetonitrile in the mobile phase while the ratio of the other two solvents remain unchanged, the retention time of OXC was slightly shortened from 4.5 to 3.0 min and the resolution between the peaks of OXC and CBZ was changed from7.2 to 3.8. When the pH value of the mobile phase was changed from 4.0 to 8.0, the resolution between the peaks of OXC and CBZ was from 6.5 to 3.2, but slight changes were found with the retention time of OXC. All things considered, a mobile phase consisting of acetonitrile, phosphate buffer (pH 6.8) and water (36:8:56, v/v) was finally selected in order to achieve optimal separation, high sensitivity and good peak shape.

Flow rates between 0.8 and 1.5 ml/min were studied. Finally, a flow rate of 1.0 ml/min was employed since it produced a reasonable retention time of OXC and acceptable resolution from its related compounds. The maximum absorption of OXC in the mobile phase was found to be at 255 and 304 nm, respectively. Since the absorbance at 255 nm was much higher than that at 304 nm, the wavelength 255 nm was chosen as the detection wavelength.

Under the proposed chromatographic conditions, OXC could be well separated from its related compounds such as synthetic starting compound, intermediate and CBZ. The retention time for OXC was observed to be about 4.0 min. Total time of analysis was less than 15 min. The chromatograms for separation of OXC from 10-methoxy-5H-dibenz[b,f]azepine, 10-methoxy-5H-dibenz[b,f]azepine-5-carboxamide and CBZ are separately shown in Fig. 2A and B, indicating good resolution between OXC and its related compounds.

3.2. Linearity

Linearity was evaluated by determining five standard working solutions of OXC ranging from 9.96 to 99.6 μg/ml, which were prepared by appropriate dilution of 99.6 μg/ml standard solution of OXC with the mobile phase. The peak area (A) and concentration of OXC (C) were subjected to regression analysis to calculate calibration equation and correlation coefficients. The regression equation was $A = 275.9 + 520.3C$ ($R = 0.9997$, $n = 5$), the linear range was from 9.96 to 99.6 μg/ml. The results show that an excellent correlation existed between the peak area and concentration of OXC.

3.3. Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were separately determined at a signal-to-noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of OXC until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations. The LOD and the LOQ of OXC were found to be 0.39 and 1.2 μg/ml, respectively.

3.4. Selectivity

The selectivity of the proposed method was challenged by forced degradation of the drug product. Tablet samples were subjected to stress conditions of light ($\sim$3000 lux), heat (105,

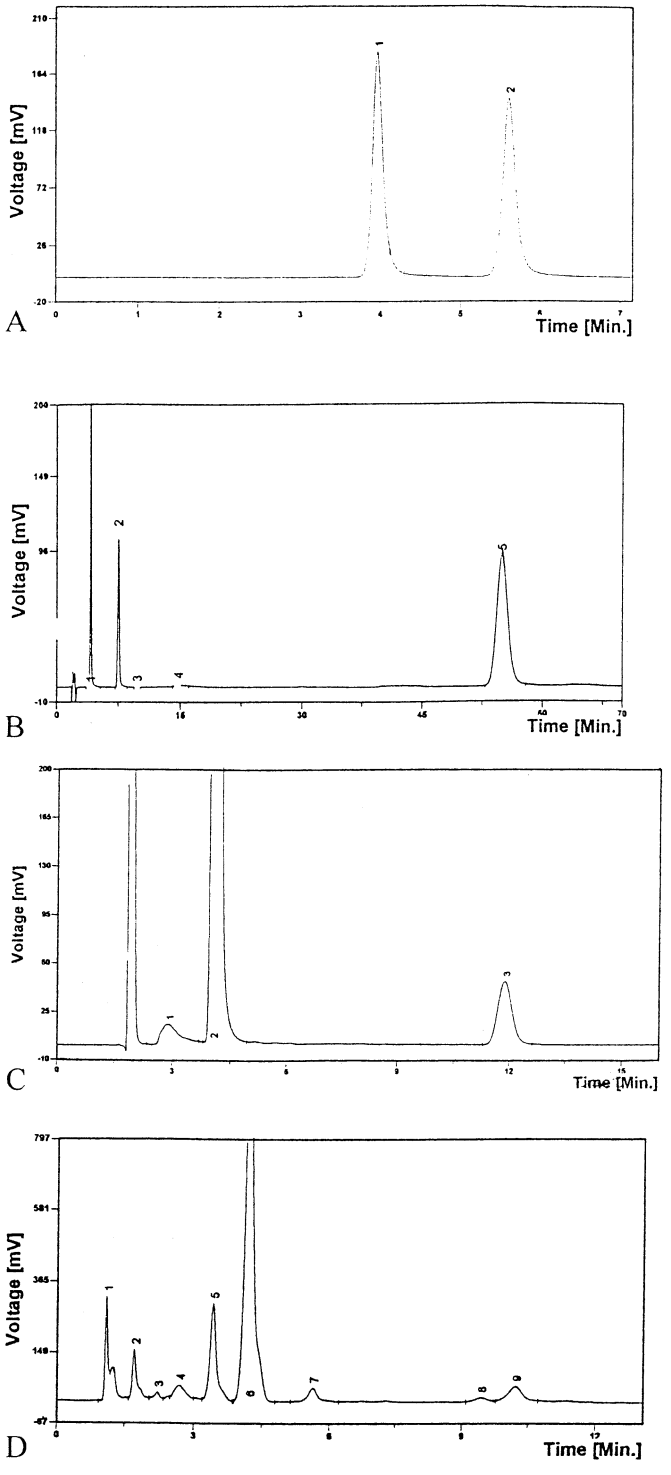
A
Voltage [mV]
Time [Min.]
B
Voltage [mV]
Time [Min.]
C
Voltage [mV]
Time [Min.]
D
Voltage [mV]
Time [Min.]

Fig. 2

200 °C), acid (6 M HCI) and base (6 M NaOH) in order to evaluate the ability of the proposed method to separate OXC from degradation products. For these studies, time period for stress test was 10 days. The proposed chromatographic conditions were found to be specific to the samples subjected to the applied stress conditions. In the case of stress by light and heat (105 °C), it was observed that rigorous stress of the samples did not cause significant degradation. Stress by high temperature (200 °C), acid and base, however, did result in significant degradation. Chromatograms of sufficiently degraded tablet samples of OXC by acid and heat (200 °C) are separately shown in Fig. 2C and D, indicating that the degradation products could be resolved from OXC. The tablet excipients were also determined to see if any interferences from the excipients existed. No peaks were observed in the chromatogram of blank sample, indicating no interferences from the excipients.

3.5. Accuracy and precision

The accuracy and precision were evaluated using a 3×3 matrix, i.e. each of the three levels of OXC standard separately at 80, 100 and 120% of label claim was performed in triplicate. Recovery was calculated to estimate the accuracy of the developed method. Mean recovery (Mean $\pm$ S.D.) and relative standard deviation (RSD) were found to be 100.1 ± 0.64 and 0.64% ($n = 9$), respectively. The within-run precision and between-run precision of the proposed method were determined by assaying the tablets in triplicate per day for consecutive 5 days. The within-run precision and between-run precision were found to be 0.6% and 1.3%, respectively.

3.6. Robustness

To evaluate method robustness a few parameters were deliberately varied. The parameters included variation of C18 columns from different manufacturers and solvents (acetonitrile) of different lots. Two analytical columns, one (Diamonsil C18 column) from Dikma (Beijing, China) and the other (Hypersil C18 column) from Elete (Dalian, China), were used during the experiment. The assay results from the two columns were 99.5 ± 0.71 and 99.7 ± 0.89% (Mean $\pm$ S.D.), respectively, indicating there is no significant difference between the assay results from the two columns. It was also found that acetonitrile of different lots from the same manufacturer had no significant influence on the determination. Insignificant differences in peak areas and less variability in retention time were observed. The RSD of less than 2.0% for peak areas and retention time were obtained, which indicated that the developed method was capable of producing results with high precision.

3.7. Analysis of the tablets

Experimental results of the amount of OXC in the tablets of three lots, expressed as the percentage of the label claim, were separately 101.0, 101.6 and 100.7% ($n = 3$). The results show that the amount of OXC in the tablets meets the requirements (90–110% of the label claim).

4. Conclusion

The developed HPLC method for the determination of OXC in the tablet formulation offers sufficient accuracy, precision, selectivity and a short run time. In conclusion, the developed

Fig. 2. Chromatogram of (A) a synthetic mixture of OXC, 10-methoxy-5H-dibenz[b,f]azepine and 10-methoxy-5H-dibenz[b,f]azepine-5-carboxamide, (B) a synthetic mixture of OXC and CBZ, (C) tablet sample forcedly degraded by HCI and (D) tablet sample forcedly degraded by heat. Peaks: (1) OXC; (2) 10-methoxy-5H-dibenz[b,f]azepine-5-carboxamide;(3) 10-methoxy-5H-dibenz[b,f] azepine; 4-carbamazepine.

method can be used for the determination of OXC in pharmaceutical formulations.

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