Solubility of Azilsartan in Methanol, Ethanol, Acetonitrile, n-Propanol, Isopropanol, Tetrahydrofuran, and Binary Solvent Mixtures between 293.15 and 333.15 K

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ABSTRACT: A precise determination method of azilsartan solubility between 293.15 and 333.15 K in several ordinary solvents and some of their aqueous mixtures was established by high-performance liquid chromatography. In all tested solvents, its solubility shows exponential growth with the increase in temperature. This trend is especially pronounced in methanol and ethanol. The order of solubility of azilsartan can be expressed as ethanol > tetrahydrofuran > ethanol/water (8/2, v/v) > methanol > methanol/water (8/2, v/v) > n-propanol > isopropanol > ethanol/Water (5/5, v/v) > acetonitrile. The solubility data of azilsartan were well correlated by the $\lambda h$ model. Moreover, the thermodynamic data including the dissolving enthalpy, entropy, and Gibbs free energy of azilsartan in each solvent were calculated which is crucial to its preparation technology study.

1. INTRODUCTION

Azilsartan (2-ethoxy-1-[(2′-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylic acid) (Figure 1), as a hypotensive drug, is a novel angiotensin II receptor antagonist that competitively blocks the binding of angiotensin to the AT1 receptor. It was first synthesized in 1995 by Kohara and approved for sale in Japan in 2012. Because of its high efficiency and less adverse effects such as dry cough compared to the similar products, azilsartan is widely applied clinically in recent years. It is reported that there are four kinds of crystalline forms of this chemical, and the crystal form, size distribution, and polymorphism could influence its pharmaceutical quality and effectiveness. Despite sufficient studies on its polymorph, the solubility information of this drug has been ignored. Indeed, multiple solvents were applied in its crystallization and recrystallization process such as isopropanol, dimethylformamide, methanol, and acetonitrile. In addition, ethanol was used as the washing solvent in the synthesis process of azilsartan to achieve the desired purity.

It is known that the choice of the solvent and its dissolving capacity have a huge impact on the drug manufacturing process efficiency. Technically, the solubility information of drugs is essential in all steps of drug discovery and development processes such as crystallization, separation, liquid extraction, and drug formulation.

In this study, the solubility of azilsartan in methanol, ethanol, acetonitrile, n-propanol, isopropanol, tetrahydrofuran, methanol/water (8/2, v/v), ethanol/water (8/2, v/v), and ethanol/water (5/5, v/v) was obtained based on the chromatographic method. The thermodynamic parameters, such as dissolved enthalpy, were calculated according to the van’t Hoff equation.

2. RESULTS AND DISCUSSION

2.1. High-Performance Liquid Chromatography. The purity of azilsartan was 99.84% determined by high-performance liquid chromatography (HPLC) with the optimized condition (Figure 2).

Figure 1. Chemical structure of azilsartan.
The solid–liquid equilibrium was described by the $\lambda h$ model which is one of the thermodynamic models proposed by Buchowski et al.\textsuperscript{16} presented as eq 2.

$$\ln\left[1 + \lambda \frac{(1 - x_A)}{x_A}\right] = \lambda h\left(\frac{1}{T} - \frac{1}{T_m}\right)$$

(2)

Here, $x$ is the mole fraction of the solute; $T$ is the experimental temperature corresponding to $x$; $T_m$ is the melting point of the solute; $\lambda$ and $h$ are the parameters of the equation.

In order to verify the uncertainty of the data, the relative deviation ($\delta$) was introduced according to eq 3. Moreover, the deviation between $x_A^{\text{calc}}$ and $x_A$ was estimated by mean deviation (MD), measuring the correlation degree of the mathematical model.

$$\delta = \frac{x_A - x_A^{\text{calc}}}{x_A}$$

(3)

where $x_A$ is the mole fraction of solute azilsartan; $x_A^{\text{calc}}$ can be calculated from eq 2.

$$\text{MD} = 100 \frac{\sum |x_{\text{calc}} - x_{\text{exp}}|}{N}$$

(4)

here $N$ is the number of experimental data; $x_{\text{exp}}$ and $x_{\text{calc}}$ are the experimental and calculated values of solubility, respectively.

The solubility data of azilsartan in methanol, ethanol, acetonitrile, n-propanol, isopropanol, tetrahydrofuran, and mixed solvents in the temperature range from 293.15 to 333.15 K are listed in Table 1 and graphically presented in Figure 4.

The data of molar fraction ($x_A$) of the solubility are close to the theoretical molar fraction $x_A^{\text{calc}}$, indicating that the solubility experimental data have good reliability and particularly instructive.

The equation parameters, the determination coefficient $R^2$, and the MD values obtained by fitting with the $\lambda h$ model are listed in Table 2.

This shows that the $\lambda h$ model fitted the data well because the value of determination coefficient $R^2$ was between 0.9524 and 0.9998, while the value of MD was between 1.218 and 10.98. Besides the $\lambda h$ model, polynomial empirical equation, Apelblat model, and Wilson model were also used to correlate the solubility of azilsartan. However, when the polynomial empirical equation was used for fitting, the calculated solubility was significantly different from the experimental value. Moreover, when the Apelblat model and the Wilson model were used for fitting, the determination coefficient $R^2$ is negative, which means the fitting cannot converge.

The solid phase of azilsartan in equilibrium with the saturated solutions was also characterized by X-ray powder diffraction analysis, and significant differences were observed among the diffraction patterns. According to the characteristic diffraction peak, three kinds of crystalline forms were definitely distinguished. Six strong diffraction peaks between 9.183° - 23.808° were observed in the X-ray powder diffractograms obtained in methanol, ethanol, acetonitrile, n-propanol, methanol/water (8/2, v/v), ethanol/water (8/2, v/v), and ethanol/water (5/5, v/v), which was defined as crystalline form I, while 10 strong diffraction peaks together with the characteristic diffraction peak at 2θ = 7.834° in isopropanol as crystalline form II and seven strong diffraction peaks together with the characteristic diffraction peak at 2θ = 22.420° in

\textbf{Figure 2.} HPLC chromatogram of azilsartan. Inset: linear relationship between the chromatogram peak area (Y) and the concentration (X) of azilsartan in methanol.

\textbf{Figure 3.} The DSC curve of azilsartan.

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tetrahydrofuran as crystalline form III (Supporting Information).

2.3. Thermodynamic Properties for Azilsartan Dissolution. In order to have an insight into the azilsartan dissolve process in each solvents, the Gibbs energy ($\Delta_{sol}G^\circ$), the dissolving enthalpy ($\Delta_{sol}H^\circ$), and the dissolving entropy ($\Delta_{sol}S^\circ$) were further studied.

The chemical potential of the solute azilsartan in the solid and liquid phases is equivalent when the dissolution equilibrium is achieved.\(^1\) It can be expressed as eq 5.

$$\mu^\circ_A(s, T) = \mu^\circ_A(1, T, \alpha_A) = \mu^\circ_A(1, T) + RT \ln y_Ax_A$$

where, $\mu^\circ_A$ is the chemical potential of solute azilsartan when the atmospheric pressure is 0.1 MPa; $\alpha_A$ is the activity of the real solution, which is defined as $\alpha_A = y_Ax_A/\gamma_A$; $y_A$ is the activity coefficient of the solute; and $x_A$ is the mole fraction of solute azilsartan.

Therefore, the relationship between the mole fraction of solute azilsartan $x_A$ and the Gibbs free energy change in the dissolution process was established when the two phases reached equilibrium at any certain temperature ($T$) as eq 6.

$$\ln y_Ax_A = \frac{\mu^\circ_A(s, T) - \mu^\circ_A(1, T)}{RT} = -\frac{\Delta_{sol}G^\circ_A(T)}{RT}$$

where $\Delta_{sol}G^\circ_A(T)$ is the Gibbs energy of dissolution of the solute azilsartan at temperature $T$.

Generally, the Gibbs energy of dissolution at temperature $T$ is given by eq 7.

$$\Delta_{sol}G^\circ_A(T) = \Delta_{sol}H^\circ_A - T\Delta_{sol}S^\circ_A$$

Therefore, the mole fraction of solute azilsartan $x_A$ can be associated with the changes of entropy and enthalpy in the dissolution process as eq 8.

$$\ln y_Ax_A = -\frac{\Delta_{sol}H^\circ_A}{RT} + \frac{\Delta_{sol}S^\circ_A}{R}$$

The activity coefficient ($y_A$) goes to 1 when the mole fraction of the solute ($x_A$) goes to zero in an ideal dilute solution. Equation 8 was simplified as eq 9. Moreover, the
relative contributions of enthalpy $\% \zeta_{\text{H}}$ and entropy $\% \zeta_{\text{TS}}$ in the dissolution process were introduced to measure the contribution of enthalpy and entropy to the change of the Gibbs free energy during the dissolution process, as eq 10 and 11.

$$\ln x_s = -\frac{\Delta_{\text{sol}} H^o_s}{RT} + \frac{\Delta_{\text{sol}} S^o_s}{R}$$  \hspace{1cm} (9)

$$\% \zeta_{\text{H}} = \frac{|\Delta_{\text{sol}} H^o_s|}{|\Delta_{\text{sol}} H^o_s| + |T \Delta_{\text{sol}} S^o_s|} \times 100$$  \hspace{1cm} (10)

$$\% \zeta_{\text{TS}} = \frac{|T \Delta_{\text{sol}} S^o_s|}{|\Delta_{\text{sol}} H^o_s| + |T \Delta_{\text{sol}} S^o_s|} \times 100$$  \hspace{1cm} (11)

The dissolving thermodynamic data of azilsartan obtained are shown in Table 3.

The dissolution of azilsartan in the selected solvents was an endothermic and entropic increase process. In the aqueous solution, the heat absorption and entropy increased with the increase in the water ratio during the dissolution process.

3. CONCLUSIONS

In this study, the liquid chromatographic method was introduced to measure the solubility of azilsartan in methanol, ethanol, acetonitrile, n-propanol, isopropanol, tetrahydrofuran, methanol/water (8/2, v/v), ethanol/water (8/2, v/v), and ethanol/water (5/5, v/v). In the single organic solvents, the molar fraction of azilsartan in methanol, ethanol, and tetrahydrofuran is much greater than it in acetonitrile, n-propanol, and isopropanol. In the mixed solvents, azilsartan has the highest solubility in ethanol/water (8/2, v/v) aqueous solutions. Moreover, its solubility decreased when the proportion of water in the mixed solvents increased. The solubility of azilsartan in nine solvents definitely increases with the increasing temperature, and the largest solubility change happened in methanol and ethanol, which could provide the theoretical basis in its recrystallization process.

4. EXPERIMENTAL SECTION

4.1. Materials. Azilsartan used in this work was provided by Shandong Xinhua Pharmaceutical Co., Ltd. Methanol, ethanol, acetonitrile, and tetrahydrofuran (chromatographic grade) were purchased from the Beijing Bellingway Technology Co., Ltd. without further purification. n-Propanol and isopropanol were obtained from Beijing Guangtong Fine Chemical Company without further processing. Deionized water (18.2 MΩ·cm$^{-1}$) was obtained from a Millipore Mili-Q Plus water system. All solution was filtered through 0.22 μm membranes before use.

4.2. Liquid Chromatographic Conditions. The purity and content analysis of azilsartan were performed on an Ulitmate 3000 HPLC and UHPLC system (America). The stationary and mobile phase were TechMate C18 ST-II (4.6 × 150 mm, 5 μm, 100 Å) and acetonitrile/water (57/43, v/v, 1 wt % glacial acetic acid), respectively. The detection wavelength was 251 nm; the flow rate was 1.0 mL·min$^{-1}$; and the injection volume was 10 μL.

4.3. Measurement of Azilsartan Solubility. An excess of azilsartan was taken in a glass vial and mixed with 10 mL of methanol, ethanol, acetonitrile, n-propanol, isopropanol, tetrahydrofuran, methanol/water (8/2, v/v), ethanol/water (8/2, v/v), and ethanol/water (5/5, v/v) to get a supersaturated solutions, respectively. Sequentially, the vial was incubated in temperature thermostatic water bath stirring at 293.15, 303.15, 313.15, 323.15, and 333.15 K for 12 h until the dissolution equilibrium was obtained, respectively. The temperature was determined by a pure solvent bottle with a thermometer inside. The uncertainty of the temperature was ±0.1 K. After another 12 h standing at the corresponding temperature, aliquots of 1.0 mL of supernatant of each vial was withdrawn by a syringe with a 0.22 μm membrane. The solution was transferred to a dried, weighed double dish, and the dish was weighed quickly to determine the mass of the solution ($m_o$) with an uncertainty of ±0.1 mg. After the solution was completely dried under nitrogen, the residue was dissolved in methanol and exactly diluted to 10 mL. Then 10 μL of the reconstituted samples was taken for HPLC analysis. All of the experiments were carried out three times simultaneously and analyzed by HPLC.

Meanwhile, the solid phase in the equilibrium with the saturated solutions was characterized by the X-ray powder diffraction at 296(2) K under Moka ray ($\lambda = 0.71073$ Å) and $\omega$-scanning method. 

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00156.

X-ray powder diffractogram of azilsartan in methanol, ethanol, acetonitrile, n-propanol, isopropanol, tetrahydrofuran, methanol/water (8/2, v/v), ethanol/water (8/2, v/v), and ethanol/water (5/5, v/v) (PDF)

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Table 3. Dissolving Thermodynamic Data of Azilsartan in Different Solvents

| solvent              | $\Delta_{\text{sol}} H^o_s$ (kJ·mol$^{-1}$) | $\Delta_{\text{sol}} S^o_s$ (J·K$^{-1}$·mol$^{-1}$) | $\Delta_{\text{sol}} G^o_s$ (kJ·mol$^{-1}$) | $\% \zeta_{\text{H}}$ | $\% \zeta_{\text{TS}}$ |
|----------------------|-------------------------------------------|-----------------------------------------------|------------------------------------------|------------------------|------------------------|
| methanol             | 12.5957                                   | 35.297                                        | 1.565                                    | 53.312                 | 46.688                 |
| ethanol              | 12.9615                                   | 36.254                                        | 1.632                                    | 53.395                 | 46.641                 |
| acetonitrile         | 26.3828                                   | 64.913                                        | 6.097                                    | 56.532                 | 43.468                 |
| n-propanol           | 26.4377                                   | 72.866                                        | 3.666                                    | 53.725                 | 46.275                 |
| isopropanol          | 27.4736                                   | 74.287                                        | 4.258                                    | 54.200                 | 45.800                 |
| tetrahydrofuran      | 8.6881                                    | 23.489                                        | 1.348                                    | 54.204                 | 45.796                 |
| methanol/water (8/2, v/v) | 20.913                                   | 55.701                                        | 3.506                                    | 54.574                 | 45.426                 |
| ethanol/water (8/2, v/v) | 16.194                                   | 45.329                                        | 2.028                                    | 53.340                 | 46.660                 |
| ethanol/water (5/5, v/v) | 34.8772                                  | 90.548                                        | 6.580                                    | 55.208                 | 44.792                 |

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