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Developmental considerations for ethanolates with regard to stability and physicochemical characterization of efonidipine hydrochloride ethanolate

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Efonidipine hydrochloride ethanolate (NZ-105) is a novel 1, 4-dihydropyridine-derivative Ca antagonist. Its chemical structure is distinctive, being a solvate composed of an equimolar ethanol adduct of efonidipine hydrochloride, and it represents one of a few cases of solvates marketed as a pharmaceutical. The research presented in this paper used methods to assess its solid state properties and included thermal analysis (thermogravimetry-differential thermal analysis; TG-DTA), Fourier-transform infrared spectroscopy (FT/IR), evolved gas analysis-mass spectrometry (EGA-MS), environmental (low-vacuum) scanning electron microscopy (E-SEM), variable temperature powder X-ray diffraction, and single-crystal X-ray structure analysis, in order to clarify the thermal behavior of the efonidipine adducts of hydrogen chloride and ethanol in the study of the thermal stability of efonidipine hydrochloride ethanolate. Upon heating, efonidipine hydrochloride ethanolate first released ethanol, and subsequently formed a decomposition product with the elimination of chloride ion. X-ray diffraction patterns and particulate forms were markedly altered after the release of ethanol, which suggested the interaction of ethanol molecules between chloride ion and efonidipine molecules within the crystal structure. Vastly different from efonidipine, the crystal structure of efonidipine hydrochloride ethanolate arranges chloride ion within a basket-type conformation formed by the bulky diphenyl and phosphate groups. This distinctive crystal structure was thought to suppress the elimination of chloride ion and to contribute significantly to the improved thermal stability of the compound.

Introduction

Characterization of an active pharmaceutical ingredient (API) in the solid state plays an important role in pharmaceutical development[1]. Crystallinity, thermal characteristics, solubility, stability, etc., offer indispensable data for closely relating physicochemical characteristics to pharmaceutical properties (bioavailability)[2-6]. Consequently, assessing these data in detail enables development of a robust API and formulation, and achieves continual quality control[7-9].

An API consisting of organic compounds generally assumes a variety of crystalline forms depending on manufacturing conditions and the ambient environment; these forms include polymorphs, salts, solvates (including hydrates) and co-crystals. Among these forms, research has continued for many years on solvates, which offer a method for improving absorption and stability of the active drug ingredient[10-13]. In the development of solvates as pharmaceuticals, the hydrates are frequently selected as a stable crystal, and consequently, case reports of solvate development containing organic solvent are rare[14]. The principal reasons include the following: (i) adduct amounts vary according to ambient temperature and partial pressure in the vicinity of the compound that make it difficult to maintain consistent quality; and (ii) toxicity risks must be considered for the solvent comprising the solvate[15]. Thus, elucidation of the physicochemical characteristics of solvates should aid selection of the crystalline form of the API, providing a remarkable contribution to advancements in pharmaceutical development in the future.

Efonidipine hydrochloride ethanolate (NZ-105), or (±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethy-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitorophenyl)-3-pyridine-carboxylate hydrochloride ethanol (**Fig. 1** and **Table 1**), comprises a dihydropyridine-derivative Ca antagonist with a phosphonate skeleton, and was discovered by Nissan Chemical Industries[16-18]. The compound is already distributed in Japan by ZERIA Pharmaceutical Co., Ltd. and Shionogi & Co. Ltd. as a film-coated tablet called "Landel®," which is characteristically released gradually in comparison to existing Ca antagonists and exhibits a longer period of activity. This active drug ingredient was initially studied for development as a hydrochloride salt without ethanol obtained through the addition of hydrochloric acid to the efonidipine acetone solution. Development continued as a hydrochloride

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ethanolate, however, because chloride ion in a hydrochloride salt without ethanol was found to be eliminated during storage[19-21]. On the other hand, efonidipine hydrochloride ethanolate showed better dissolution behavior[22] and absorption[23] than that of its free form. As described above, due to concerns about quality control and toxicity risks, solvates tend to be avoided in pharmaceutical development, and reports of development cases are rare. The research described in this paper, therefore, studied efonidipine hydrochloride ethanolate as a rare solvate pharmaceutical through stability testing with respect to its thermal behavior and accelerated conditions for its adducts with ethanol and chloride ion. The authors also determined the crystal structures of efonidipine hydrochloride ethanolate and efonidipine free form, and studied their differences with respect to thermal stability.

Figure 1. Chemical structure of efonidipine hydrochloride ethanolate (racemate).

Table 1. Physicochemical properties of efonidipine hydrochloride ethanolate.

Experimental

2-1. Materials

Efonidipine hydrochloride ethanolate (Lot No. EFH-001) and efonidipine (Lot No. SM-00126-131) were supplied by Nissan Chemical Industries, both are racemate. All other reagents used were of reagent grade.

2-2. Thermodynamic characterization of efonidipine hydrochloride ethanolate

2-2-1. Thermal analysis

Analyses of the thermogravimetric (TG) curve and differential thermal analysis (DTA) curve were conducted using a Thermo Plus TG-8120 (Rigaku Corporation, Tokyo, Japan). For analysis, approximately 5 to 10 mg of efonidipine hydrochloride ethanolate was weighed on an aluminum pan. Analysis was performed for the temperature range from room temperature to around 300˚C at a scan rate of 5˚C/min under an air atmosphere flow (50 mL/min).

2-2-2. FT/IR spectroscopy

Infrared (IR) absorption spectra were measured according to the potassium bromide disk method using an IRAffinity-1 (Shimadzu Corporation, Kyoto, Japan). Samples were analyzed after placement for 10 min in an oven set to the respective temperature and then left at room temperature.

2-2-3. Evolved gas analysis-mass spectrometry (EGA-MS)

The analytical apparatus comprised a vertical heating furnace (PY-2010i; FRONTIER LAB, Fukushima, Japan) connected to a gas chromatograph (GC7890A; Agilent Technologies, Tokyo, Japan). A mass spectrometer (GCT Premier; Waters, Tokyo, Japan) was used as the detector.

A platinum crucible containing approximately 100 µg of efonidipine hydrochloride ethanolate was placed in a thermolysis furnace and heated at 5˚C/min from 80˚C to 180˚C. Evolved gas was passed through an inert column and was introduced in real time to the gas chromatograph-mass spectrometer (GC-MS). A DB-1 LTM inert column (0.18 mm × 20 m × 0.40 µm: Agilent Technologies, Tokyo, Japan) was used to introduce the gas from the gas chromatograph (GC) to the mass spectrometer (MS). The carrier gas was He (flow rate 0.5 mL/min), the split ratio was 1/5, the column oven was 50˚C and the injection port temperature was 300˚C. Electron ionization (EI) was employed for the MS ionization method.

2-2-4. Environmental (low-vacuum) scanning electron microscope (E-SEM)

In separate glass Petri dishes, 1 g of efonidipine hydrochloride ethanolate was placed and heated for 10 min at 100˚C or 130˚C, and was then left to stand to return to room temperature. These specimens were observed at room temperature with respect to sample form and surface conditions using low-vacuum scanning electron microscopy (Quanta 200; FEI, Tokyo, Japan).

2-2-5. Variable temperature powder X-ray diffraction

Powder X-ray diffraction (PXRD) patterns at elevated temperatures were obtained with a PXRD apparatus (X'pert pro Multi-Purpose Diffractometer; PANalytical, Tokyo, Japan). A Cu-Kα beam source (λ = 1.5418 Å) was employed for analyzing a diffraction angle range of 3˚ to 25˚ at a voltage of 45 kV and a current of 40 mA. Samples were set and the temperature was elevated at a rate of 5˚C/min for measurement at room temperature, 50˚C, 100˚C, 125˚C, 135˚C, 145˚C, 150˚C and 160˚C and holding these respective measurement temperatures constant for 5 min.

2-2-6. Stability test (Accelerated conditions)

A total of 50 g of efonidipine hydrochloride ethanolate was placed in a polyethylene bag (150 mm × 250 mm), and the bag was sealed and stored in a 40˚C and 75% relative humidity (RH) environment. After 1, 3 or 6 months of storage, stability analysis was conducted for related substances (high performance liquid chromatography; HPLC), water content (Karl Fischer; KF), hydrochloride (titration) and ethanol (GC). Other detailed experimental conditions are described in **Supporting information**.

2-3. Single-crystal X-ray structure analysis

For the preparation of efonidipine hydrochloride ethanolate single crystals, ethanol was added to efonidipine hydrochloride ethanolate, which was slowly dissolved at approximately 70˚C to 80˚C, and was then stored for 2 days at room temperature. Precipitated crystals were used. The obtained crystals were yellow and prism.

For preparation of efonidipine single crystals, methanol was added to efonidipine, which was slowly dissolved at approximately 70˚C to 80˚C, and was then stored for 2 days at room temperature. Precipitated crystals were used. The obtained crystals were yellow and prism.

Single crystal X-ray diffraction was collected at 296 K on a Bruker AXS SMART APEXII ULTRA diffractometer using monochromated Cu-Kα radiation (λ = 1.54178 Å) by a HELIOS multilayer optics. Absorption corrections were made with the program SADABS[24]. The structures were solved by the direct methods using a SHELXS-97[25] and refined by full-matrix least-

squares on F^2 using SHELXL-97[25]. Data were graphed using the Mercury program (Cambridge Crystallographic Data Centre, CCDC) and ChemBio3D Ultra 12.0 (CambridgeSoft, PerkinElmer, Tokyo, Japan).

Results and discussion

3-1. Thermal behavior and degradation of efonidipine hydrochloride ethanolate

The TG-DTA curve of efonidipine hydrochloride ethanolate is shown in **Fig. 2**. The results of TG analysis revealed loss of mass from around 80˚C in two steps; first, up to around 140˚C for demarcation, and then up to 160˚C (-6.25% and -5.11%, respectively). Characteristic thermal absorption peaks with maxima at around 132˚C and 156˚C were detected on DTA analysis, followed by observation of small exothermic peaks originating from degradation of efonidipine hydrochloride ethanolate.

EGA-MS was employed to identify volatile components in the two-step loss of mass observed under TG analysis. EGA-MS has been in the spotlight in recent years for the assessment of decomposition products in materials and can determine reaction products and eliminated components under elevated temperature in real time[26, 27]. From the results of EGA-MS analysis, the volatile component around 132˚C, whose desolvation starts at around 80˚C, was determined to be ethanol (EI-MS m/z 45.046). The volatile components at 156˚C and above were considered to be chloride ion and benzyl chloride generated through thermolysis of efonidipine hydrochloride ethanolate (EI-MS m/z 35.975 and m/z 126.023, respectively; **Fig. 3**).

In order to clarify the chemical change to solid samples consisting of efonidipine hydrochloride ethanolate whose volatile components were thought to be eliminated through heating, IR absorption spectra of samples heat-treated at the respective temperatures were analyzed (**Fig. 4**). No changes were observed with heating at any temperature for carbonyl (1705 cm^{-1}), nitro (1523 cm^{-1}) and phosphate (1248 cm^{-1}) groups characterizing efonidipine hydrochloride ethanolate, no changes were observed on heating at any temperature, but the absorption peak associated with the stretching vibration (around 2324-2356 cm^{-1}) of quaternary ammonium that forms a salt structure disappeared at 160˚C and above. This change corresponded with the results of EGA-MS, in which generation and elimination of benzyl chloride was observed. Efonidipine hydrochloride ethanolate thermolysis, or the degradation pathway according to the formation of benzyl chloride is shown in **Fig. S1** (see **Supporting Information**).

Accordingly, interactions between protonated efonidipine cations, chloride ions and ethanol molecules appear to have an influence on the thermal stability of efonidipine hydrochloride ethanolate.

Figure 2. TG-DTA curve of efonidipine hydrochloride ethanolate.

Figure 3. EGA-MS spectra indicating evolved gases from efonidipine hydrochloride ethanolate during heating.

Figure 4. IR spectra of efonidipine hydrochloride ethanolate after heating at various temperatures.

3-2. Changes in particle appearance and crystalline state of efonidipine hydrochloride ethanolate during heating

The change in appearance of efonidipine hydrochloride ethanolate particles after heat treatment is shown in **Fig. 5**. The particle size of efonidipine hydrochloride ethanolate powder ranged between 10 and 30 μm, and the mean particle size ranged between 23 and 30 μm (**Table S1**). Particles at room temperature under unheated conditions had a block-like appearance. When heated to around 100˚C, although the particle corners became slightly rounded, the particles retained their overall form. At around 130˚C, when ethanol became volatile, as described in the previous section, the particles melted or dissolved in the released ethanol.

Variable temperature PXRD was used to analyze the change in the crystalline state of efonidipine hydrochloride ethanolate during temperature elevation (**Fig. 6**). The X-ray diffraction peaks inherent to efonidipine hydrochloride ethanolate for 2θ = around 11.5˚, 16˚ and 21˚ shifted slightly to lower angles at around 130˚C to 140˚C. Consequently, expansion of the crystal lattice in conjunction with an elevated ambient temperature from heating was inferred[28, 29]. The intensity of diffraction peaks clearly decreased at around 140˚C, and a nearly halo pattern was observed at 150˚C and above. The diffraction pattern at 150˚C revealed faint diffraction peaks, which suggested the presence of a contracted crystal structure after the volatile components were eliminated from efonidipine hydrochloride ethanolate. As the thermal analysis results showed that ethanol volatilized at around 130˚C to 140˚C, ethanol molecules were inferred to serve an important role in maintaining crystal structure.

Solvates are generally categorized broadly between stoichiometric solvates and non-stoichiometric solvates according to the amount of solvent added[30, 31]. For a stoichiometric solvate, the solvent ordinarily plays an important role in the crystal structure and forms a strong interactive network with other molecules. Thus, after the solvent is eliminated, transition to a different crystal shape or amorphous state occurs. The solvent for a non-stoichiometric solvate is frequently present in a format where space in a channel structure formed within the crystal structure is filled. Thus, interaction between the solvent and other molecules is relatively weak[32-35]. For efonidipine hydrochloride ethanolate, ethanol molecules are present in an equimolar ratio to efonidipine hydrochloride. After elimination of ethanol at around 140˚C, the PXRD peak intensities decreased significantly while crystalline diffraction peaks remained. This suggests more of a stoichiometric solvate whose solvent molecules are present as members of an interactive network.

Figure 5. SEM images of efonidipine hydrochloride ethanolate.

Figure 6. Changes in variable temperature PXRD patterns of efonidipine hydrochloride ethanolate.

Stability tests were conducted for efonidipine hydrochloride ethanolate under accelerated conditions of 40˚C and 75% RH in order to study its stability as a pharmaceutical (**Table 2**). Efonidipine hydrochloride ethanolate was found to remain physically stable with no changes in appearance observed for 6 months, and with no volatile release (Table 2). As can be expected from the robustly maintained crystal structure, no problems were noted for assay values or related substances. The powder was confirmed to have an extremely stable crystalline structure as an API.

Table 2. Stability test results for efonidipine hydrochloride ethanolate.

3-3. Crystal structure analysis of efonidipine hydrochloride ethanolate and its free form

The single crystal structure of efonidipine hydrochloride ethanolate is shown in **Fig. 7a**. The crystal lattice of efonidipine hydrochloride ethanolate (racemate) is triclinic and efonidipine hydrochloride ethanolate belongs to space group $P\bar{1}$. The R-value is 4.77%, lower than the reported value (R-value: 8.1%)[20]. The actual model and calculated space-filling model matched well. As reported, the structure of the compound is quaternary ammonium with a proton shifted to the nitrogen atom, and was thus demonstrated to be a hydrochloride. Detailed crystallographic parameters are shown in **Table 3**.

Within the efonidipine hydrochloride ethanolate crystal structure, the torsion angles of Φ_1[C9-C8-C19-O7], Φ_1[C8-C7-C1-C6], Φ_1[C8-C7-C1-C2] and Φ_1[C10-C11-P1-O3] were respectively 179.0(2)°, 67.4(2)°, -112.2(2)° and -80.7(2)°. Thus, the carboxyl group was in a synperiplanar (SP) environment relative to the double bonds of the dihydropyridine (DHP) ring; consequently, there was no torsion. The phenyl group was positioned exactly where the DHP ring was divided equally in two. This environment was inferred to minimize steric hindrance between the 3-carboxyl group and 5-phosphate ester group. The DHP ring had a boat form in the efonidipine hydrochloride ethanolate structure. Thus, the magnitude of torsion of the DHP ring peaked at C12, and the phenyl group at C12 was in a pseudo-axial direction. These characteristics of the steric structure are similar to those of DHP-1,4-dicarboxylates[36, 37], such as nifedipine, nisoldipine[38-40] and felodipine[41]. On the other hand, the P-C bond of the 1,3,2-dioxa-phosphorinane ring was in the axial direction, and adopted a chair conformation. Consequently, a cavity in the basket form was produced by the phosphate ester group and diphenyl group, large functional groups within the structure. It was inferred that these functional groups facilitated the incorporation of chloride ion and ethanol molecules. This type of structure was not observed among similar DHP-1,4-dicarboxylates, but rather was distinctive for efonidipine hydrochloride ethanolate. Although efonidipine hydrochloride ethanolate is a DHP Ca antagonist, recent pharmacological evidence indicates that it can block both L-type and T-type calcium channels, unlike other DHP antagonists. The drug is gaining attention as a candidate therapeutic agent[42, 43]. The structural differences reported here for efonidipine hydrochloride ethanolate compared to other Ca antagonists are likely involved in the observed differences in pharmacological action.

Next, attention toward the volatile components within the efonidipine hydrochloride ethanolate crystal structure revealed that chloride ions are linked to the efonidipine cation by strong N-H^+···Cl^- hydrogen bonds and located inside the basket-type conformation formed by the efonidipine molecules (**Fig. 7d**). Although the ethanol molecules are disordered, they might be hydrogen bonded with the phosphate ester portion of efonidipine molecule in the crystal structure of its hydrochloride ethanolate (O ···O=P, about 2.7 Å).

Single crystals of the R and S forms were both obtained from efonidipine which is also originally racemate, but favorable results from crystal structure analysis were obtained only for the R form (**Fig. 7b**). Overlapping the conformation of the efonidipine molecule showed that the crystal structures of the "linear" free form (R form) was changed by flipping the bulky diphenyl group into the "basket" form. Efonidipine hydrochloride ethanolate (racemate) can accept a guest molecule (**Fig. 7c**). The API form in the initial development stage of the drug was the hydrochloride salt without ethanol, but there were problems with physical stability because chloride ion eliminated during storage; furthermore, there were problems in obtaining the crystal structure of the drug. On the other hand, the present research clarified that efonidipine hydrochloride ethanolate, in contrast to the general notion of solvates, suppressed the release of chloride ion following the addition of an ethanol molecule to the crystal structure, resulting in improved physical stability. As the degradation pathway of this system involves the generation of benzyl chloride, it was also verified from the crystal structure that the suppression of chloride ion elimination significantly improved chemical stability.

Figure 7. Crystal structures of efonidipine hydrochloride ethanolate and its free form.

Table 3. Crystallographic parameters of efonidipine hydrochloride ethanolate and its free form.

Conclusion

This research focused on the relationship between the physical characteristics (mainly in relation to thermal behavior) and the crystal structure of efonidipine hydrochloride ethanolate, one of the few solvates deployed to date as a pharmaceutical. The phosphonate group that represents the characteristic chemical structure of efonidipine hydrochloride ethanolate adopted an equatorial orientation relative to the DHP ring to avoid steric hindrance. As a consequence of the bulky phosphonate and diphenyl groups, it was clarified that a cavity was created within the efonidipine hydrochloride ethanolate structure to incorporate chloride, forming a stable solvate. This characteristic structure has not been observed for the

analogous DHP calcium drug-form, and the difference in structure contributes to the observed difference in efficacy. There are few data on ethanolate pharmaceuticals. In the case of efonidipine hydrochloride ethanolate, however, ethanol contained in the crystal structure might contribute to the physical stability of the drug. After ethanol volatilized at around 130˚C, chloride ion was released and eliminated, and caused a degradation reaction. The ethanol in the efonidipine hydrochloride ethanolate structure, therefore, might be indispensable for the retention of chloride ion in the crystal structure and for improved thermal stability.

The knowledge gained by this research is expected to diversify the selection for active drug ingredients in pharmaceutical development work, and to be useful in formulation studies, a field that has become increasingly sophisticated in recent years.

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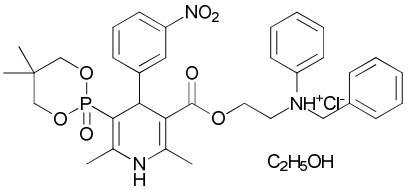

Figure 1. Chemical structure of efonidipine hydrochloride ethanolate (racemate).

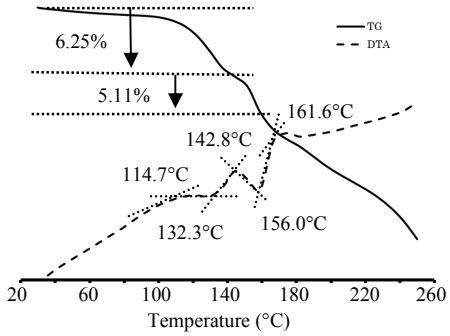

Figure 2. TG-DTA curve for efonidipine hydrochloride ethanolate. Arrows indicate each loss on TG. Temperatures were calculated from intersection of tangent lines.

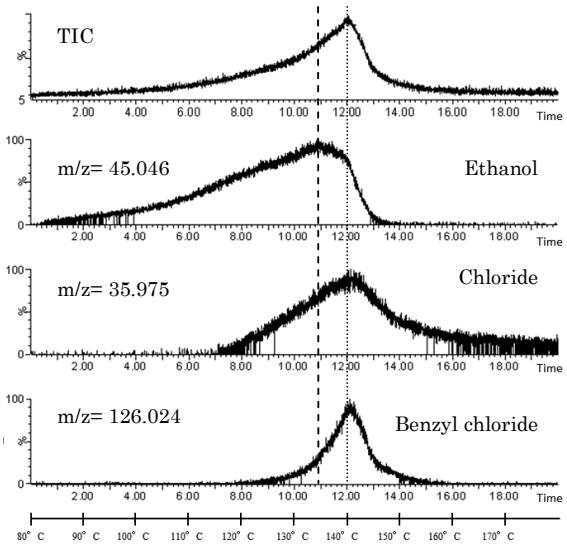

Figure 3. EGA-MS spectra indicating evolved gases from efonidipine hydrochloride ethanolate during heating. Dashed line indicates peak for ethanol and dotted line indicates that for chloride ion.

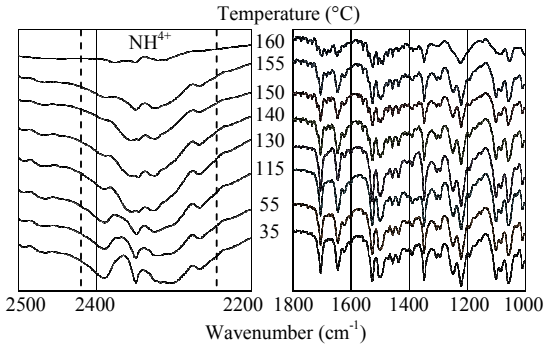

Figure 4. IR spectra of efonidipine hydrochloride ethanolate after heating at various temperatures. Two vertical dashed lines show the region of NH^{4+} stretching vibration.

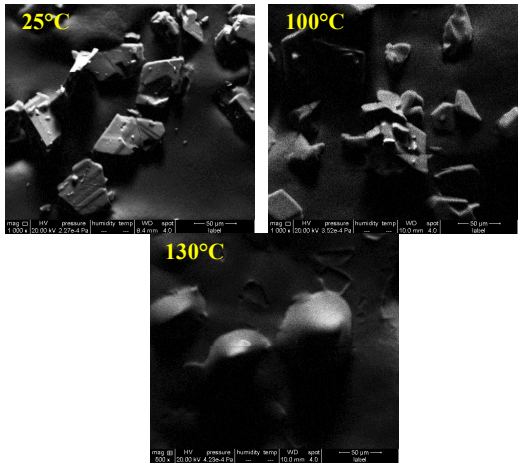

Figure 5. SEM images of efonidipine hydrochloride ethanolate during heating.

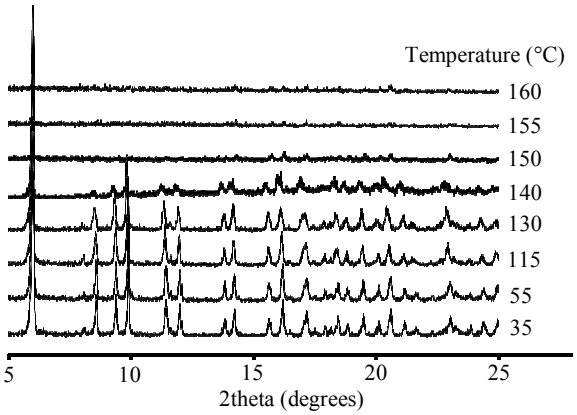

Figure 6. Changes in variable temperature PXRD patterns of efonidipine hydrochloride ethanolate.

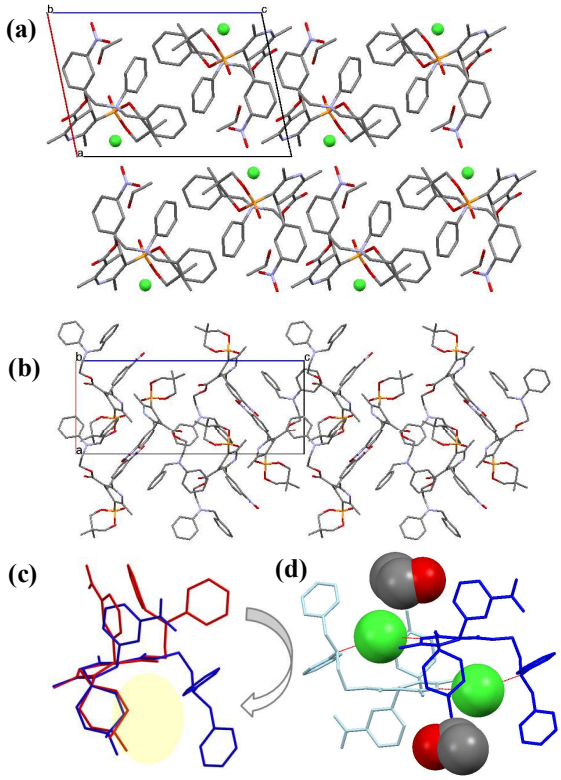

Figure 7. Crystal structures of efonidipine hydrochloride ethanolate (racemate) (a) and its free form ((*R*)-form) (b). ; (b) Single crystals of the R and S forms were both obtained from efonidipine which is also originally racemate, but favorable results from crystal structure analysis were obtained only for the R form. (c) Flipping diagram of efonidipine molecule in crystal structure of efonidipine hydrochloride ethanolate (blue) and efonidipine (red). (d) Hydrogen bonding network of efonidipine hydrochloride ethanolate. Red dashed lines indicate intermolecular hydrogen bonds.

1 **Table 1. Physicochemical properties of efonidipine hydrochloride ethanolate.**

Molecular formula	$C_{34}H_{38}N_3O_7P{\cdot}HCl{\cdot}C_2H_6O$
Molecular weight	714.17
Melting point (°C)	151
Dissociation constant pKa	3.27
Distribution constant	≥ 1000
Solubility (mg/mL)	
Water	< 0.1
Aqueous buffer pH 1.2	< 0.1
Aqueous buffer pH 4.0	< 0.1
Aqueous buffer pH 4.0 – 12.0	< 0.1
Ethanol	4.14 ± 0.03
Methanol	29.6 ± 0.15
Formic acid	588.2
N,N-Dimethylformamide	312.5

2

3

Table 2. Stability test results for efonidipine hydrochloride ethanolate under accelerated conditions.

	Initial	1 month	3 months	6 months
Appearance	Powder crystal	Powder crystal	Powder crystal	Powder crystal
Related substance (%)	0.04	0.05	0.05	0.04
HCl content (%)	5.10	5.11	5.08	5.06
EtOH content (%)	6.42	6.25	6.12	6.23
Assay (%)	100.0	99.9	100.2	100.3

Table 3. Crystallographic parameters of efonidipine hydrochloride ethanolate and its free form.

	Efonidipine hydrochloride ethanolate	Efonidipine
Molecular formula	$C_{34}H_{38}N_3O_7P{\cdot}HCl{\cdot}C_2H_6O$	$C_{34}H_{38}N_3O_7P$
Molecular weight	714.17	631.64
Temperature (K)	296	296
Crystal color, habit	Yellow, Prism	Yellow, Prism
Crystal system	Triclinic	Orthorhombic
Space group	$P\bar{1}$	$P2_12_12_1$
Lattice parameters (Å, °)		
a =	11.4763(11)	9.9296(5)
b =	11.9281(12)	13.1794(7)
c =	15.1054(15)	24.2673(12)
α =	87.156(5)	90.00
β =	78.155(5)	90.00
γ =	64.918(4)	90.00
Volume (Å^3)	1831.3(3)	3175.8(3)
Z	2	4
D_{calcd} (Mg m^{-3})	1.295	1.321
Reflections collected	25692	46156
Independent reflections	5958	5382
R_{int}	0.0224	0.0491
Absolute structure parameter	-	0.01(3)
Goodness-of-fit on F^2	1.086	0.858
Final R indices [$I > 2\sigma(I)$]	R_1=0.0433 wR_2=0.1418	R_1=0.0418 wR_2=0.1066
R indices (all data)	R_1=0.0477 wR_2=0.1533	R_1=0.0615 wR_2=0.1349