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RESEARCH ARTICLE

Preformulation evaluation of AZD1305, an oxabispidine intended for oral and intravenous treatment

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Abstract

Aim: AZD1305 is a novel, water-soluble investigational antiarrhythmic agent for restoration and maintenance of sinus rhythm in atrial fibrillation patients. The present studies were performed to evaluate the possibility for further development of the compound.

Methods: A set of technical approaches were used, including X-ray powder diffractometry, differential scanning calorimetry, thermogravimetrical analysis, dynamic vapor sorption, scanning electron microscopy, salt screen, and liquid chromatography.

Results: AZD1305 is a crystalline oxabispidine and its neutral form is a base with a pK_a of 9.9. The substance degrades with higher temperature and lower pH. The free base of the solid substance is stable at 25°C (closed container), 40°C/75% relative humidity (open container), and at 50°C (closed container) for at least 3 months. The free base of AZD1305 is polymorphic with two known forms. Both forms are non-hygroscopic ansolvates with melting points of approximately 90°C. No salt was found with overall improved properties. The substance had a strong odor, which was reduced by increased particle size.

Conclusions: The free base of AZD1305 seemed to be the most suitable agent for product development even though it has a fairly low melting point and occurred as two different crystal forms. Form B was the most stable thermodynamically in the temperature interval of interest.

Keywords: Drug delivery, early development, odor, polymorphs, salt-screen, smell, solid-state, stability

Introduction

During the preformulation phase, the availability of a compound is limited and the quality sometimes poor[1]. The first step in development work is to establish analytical methods. Most drugs absorb light in the UV region as they are generally aromatic and/or contain other chromophores. In order to follow drug stability, in both solution and solid state, it is necessary to have suitable, controlled equipment together with validated analytical methods, such as well-documented liquid chromatography (LC) approaches, which are the principal methods of choice in the pharmaceutical industry today[2,3]. The results from stability studies give hints about how to treat the bulk substance as well as inform on formulation approaches, excipients, stabilizers, and packaging requirements for the compound and/or on formulation of the compound and possible future product[4]. Drug degradation occurs mainly through hydrolysis, oxidation, and photolysis, caused by e.g. trace metal catalysis from the synthesis, oxygen or humidity in the air, or light exposure[5–8]. Conditions affecting the stability of the formulation include temperature, light, pH (liquids), and/or relative humidity (RH; solid state)[9–12]. The acidic and basic nature of the molecule can be predicted from the functional groups of the compound and an initial estimation of the pK_a(s) can be made before confirming analytically[13]. The most widely used software is ACDLabs (Advanced Chemistry Development)[14]. As a minimum requirement during early formulation development, the solubility and pK_a must be determined[1,15]. It is well known

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that the ionization state and the solubility of a drug affect absorption, distribution, metabolism, and excretion[16,17]. Of special importance is the intrinsic solubility (S_0), i.e. the solubility of the drug when unionized[1,15]. The values of pK_a and S_0 dictate the ease with which formulations are obtained for oral gavage and intravenous (i.v.) injection studies in animals; how solubility varies with changes in pH; and whether or not a salt formation can be achieved to increase bioavailability and/or stability and overall powder properties[18–22].

The issue of possible drug polymorphism, in its crystalline state, must be explored since polymorphs may have appreciable differences in physicochemical properties such as solubility and melting points[23–26]. Of concern are the relative stabilities and solubilities of crystalline modifications[27]. The compound with the highest melting point is generally the most stable thermodynamically form and often the most preferred for further development[28–30]. Polymorphs can transform to other solid-state forms during manufacturing processes and storage, often related to humidity, temperature, and impurities/additives[31–33]. In general the other, metastable forms, convert to the most stable form with time. The solubility and melting point are related to each other *via* the enthalpy of fusion, i.e. the heat generated during melting or fusion. A strong crystal lattice leads to a high melting point and a high heat of fusion, but also to a lower solubility (even if other factors also contribute to the solubility). In addition, the bonds between crystals affect the melting point and thus also the solubility[22].

AZD1305 is an investigational novel, water-soluble antiarrhythmic oxabispidine developed for restoration and maintenance of normal sinus rhythm (Figure 1). AZD1305 can be characterized as a combined ion-channel blocker that delays repolarization. The original objective[34] of the project was to bring AZD1305 to the market for the management of atrial fibrillation and flutter. Two separate formulations were planned: a parenteral formulation to convert patients into a sine rhythm, and an oral formulation to maintain the patients in the attained sine rhythm. For the maintenance indication, prediction of a short elimination half-life in human indicated that an extended release (ER) formulation was required. The permeability over the human colon, using an Ussing chamber set-up, was about 10×10^{-6} cm/s at 10 μM. The therapeutic dose was expected to be about 250–500 mg/day. The aim of the present report was to determine whether AZD1305 had the fundamental physicochemical properties to support further development.

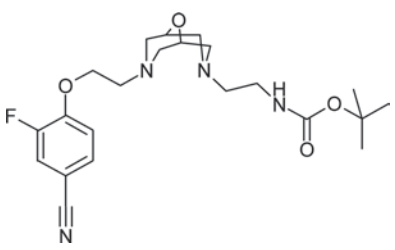

Figure 1. The figure shows the structure of the drug substance, AZD1305.

Material and methods

Test compound and chemicals

AZD1305 ($C_{22}FN_4O_4H_{31}$, with a mass of 434.5 g/mol) was synthesized at AstraZeneca R&D (Mölndal, Sweden). Acetonitrile, methanol, *n*-heptane, trifluoroacetic acid, and acetone were purchased from Merck (Darmstadt, Germany). Chloroform was obtained from Sigma Chemicals (St Louis, MO). Tetrahydrofuran (THF) and 2-propanol were from Rathburn Chemicals (Walkerburn, Scotland). Ethyl methyl ketone (EMK), methyl isobutyl ketone (MIBK), and ethylacetate were purchased from Scarlau Chemie (Sentmenat, Spain) and ethanol was obtained from Kemetyl (Haninge, Sweden). Isooctane and mannitol were purchased from Riedel-de Haën (Sigma-Aldrich Laborchemikalies, Seelze, Germany). HPMC 60SH50 was purchased from Shin-Etsu Chemicals (Tokyo, Japan). All counter-ions used in the salt screen (see below) were purchased from Sigma-Aldrich (Steinheim, Germany). The material for the LC methods was obtained from the following companies: acetic acid (Merck), ammonium acetate (Scharkau, Sentmenat, Spain), acetonitrile (Fischer Scientific, Loughborough, UK), sodium dihydrogen phosphate (Merck), di-sodium hydrogen phosphate (Merck), and orthophosphoric acid 85% (Scharlau, Sentmenat, Spain).

XRPD

X-ray powder diffractometry (XRPD) experiments were performed on a D8 Advance diffractometer (Bruxer AXS GmbH, Karlsruhe, Germany) with Bragg-Brentano geometry, equipped with a VÅNTEC-1 position-sensitive detector. Nickel-filtered Cu K_α radiation was used. The samples, approximately 10 mg, were mounted on a zero-background holder (silicon crystal). Data were collected using continuous scan mode in the range 1–50° 2θ, with a step size of 0.017° and a step time of 0.5 s. A variable (V20) divergence slit and a detector slit of 12 mm, corresponding to a 3.47° wide detector window, were applied.

DSC

Differential scanning calorimetry (DSC) analysis was performed using a DSC Q1000 (TA Instruments, New Castle, DE). The temperature and heat flow were calibrated using indium. Experiments were run between 25 and 200 or 250°C with a heating rate of 10°C/min in a dry purge of nitrogen gas (50 mL/min). The sample was analyzed in an aluminum pan with closed lid; however, an approximately 1-mm wide hole had been made in the lid with a needle.

TGA

Thermogravimetrical analysis (TGA) analysis was performed using a TGA Q500 (TA Instruments). The temperature was calibrated using the Curie points of alumel

alloy and nickel. The balance was calibrated using 100 and 1000 mg standard weights. The samples were heated from room temperature to 220°C with a heating rate of 10°C/min in a dry purge of nitrogen gas (90 mL/min).

DVS

Water sorption measurements were carried out using a commercial instrument: dynamic vapor sorption (DVS) (DVS-1; Scientific & Medical Products Ltd, Manchester, UK). The automated instrument measures the uptake or loss of water vapor gravimetrically using a Cahn D200 recording ultra-microbalance, in a symmetric arrangement of sample and reference weighing pan, with a mass resolution of 0.1 μg.

SEM

Morphological evaluation of AZD1305 was conducted through scanning electron microscopy (SEM; FEI Company, Ekerö, Sweden) following gold coating.

Small-scale wet granulation

Small equal amounts (approximately 75 mg) of AZD1305 (form B) and HPMC 60SH50 were mixed and 5 drops of ethanol or 1 drop water or water/ethanol mixture (50/50) were added and kneaded in a mortar. The wet masses were dried in oven at 60°C for 12 h. These samples were compared with physical mixtures with and without drying in oven in 60°C for 12 h. The physical mixtures were not kneaded.

pK_a measurement

The measurement was performed essentially with the same equipment and method as earlier described by Wan et al.[13]. The method is based on a capillary electrophoresis (CE) and mass spectrometry (MS) set-up. All CE separations were performed using a CE instrument (HPCE^{3D}; Agilent Technologies) coupled on-line with an 1100 series LC/MSD trap (SL) and a binary LC pump. Untreated fused-silica capillaries (Skandinaviska GeneTec AB, Sweden) were used. The capillary was thermostated at 25°C by utilizing an external water bath connected to the sample tray. The pH of the buffers was measured with a pH meter (PHM240, pH ion/meter; MeterLab, France).

Solubility

The solubility of AZD1305 in aqueous solutions, at different pH, was determined by adding an excess of the crystalline drug into the solvent. The suspensions were stirred on a magnetic stirrer at 22°C for 24 h, filtered (cut-off 0.22 μm, Millex-GV, polyvinylidene fluoride; Millipore, Carrigtwohill, Co Cork, Ireland) and the content of dissolved AZD1305 was analyzed by high-performance liquid chromatography (HPLC) as described below.

Salt screen

Salt screening was carried out in small scale (2–3 mg of AZD1305 per experiment) using 30 pharmaceutically relevant counter-ions from the literature. The solvents were evaporated under a gentle nitrogen purge on a shaking table. The obtained solids were examined for crystallinity by microscopy using an Olympus microscope and crossed polarizer (Olympus IX71; Olympus Optical Co Ltd, Japan). The crystalline samples were then analyzed for proof of salt formation using Raman microscopy (Labram HR 800 Raman microscope; Jobin Yvon/Horiba, New Jersey, USA). Recrystallizations were attempted in similar conditions from methanol, ethanol, ethyl acetate, and acetonitrile.

Slurries

The samples (about 40 mg) were suspended in about 500 μL of water, ethanol, methanol, 2-propanol, isooctane, *n*-heptane, ethylacetate, acetone, acetonitrile, chloroform, THF, EMK, or MIBK. They were stored at room temperature for 2 weeks. Small amounts of samples of suspension were withdrawn at suitable time intervals, dried at room temperature, and investigated with XRPD and DSC. For the salts, about 20 mg were used for each solvent, at each occasion.

Measurements of degradation products and organic impurities

Analytical equipment

Degradation products and impurities were analyzed with an Agilent 1100 HPLC system (Agilent Technologies, USA). A quaternary pump together with a degasser was coupled to an auto-sampler with a thermostatic sample tray. The column was thermostated in a column oven. The separated samples were detected with a diode array detector. For identification purpose, an 1100 MSD detector was used.

LC method

The separation column used was an ACE 3 CN 100 × 4.6 mm column from Advanced Chromatography Technologies (Scantec Lab, Partille, Sweden). The flow rate was 1 mL/min and the column temperature was 25°C. A linear gradient was run according to the following scheme: 0–5 min 20% A, 5–35 min 20–90% A, 35–40 min 90% A. Solvent A consisted of acetonitrile and solvent B consisted of water solution of ammonium acetate pH 4.5, I 0.02 M. Twenty microliters of 0.3 mg/mL of AZD1305 solved in mobile phase or buffer was injected on the LC-system. Twenty microliters of sample was used as injection volume. The detection wavelength was 254 nm. The LC method was fully validated (AstraZeneca, data on file).

Photostability

To decide on whether an amber-colored container is required or whether a color masking dye should be used during formulation development, a light stability procedure was performed. The photostability of AZD1305 was tested using a sunset CPS$^+$ apparatus equipped with a xenon lamp and a cooling aggregate. A quartz glass filter dish with selectively reflecting coating was installed to

transmit UV (320–400 nm) and visible radiation (400–800 nm) but reflect infrared radiation. A study was performed where AZD1305 drug substance was exposed to light with an overall illumination of not less than 1.2 million lux hours and near UV energy of 250 Wh/m^2 for 24 h.

Results and discussion

Solid-state properties of the free base of AZD1305

The free base of AZD1305 was obtained in two different crystal forms (form A and form B), which were characterized by XRPD (Table 1, Figure 2).

Analysis with DSC showed a melting point onset around 90°C for both forms, indicating that they might be energetically similar (Figure 3). However, the melting enthalpy was slightly higher for form B.

DVS experiments showed that both forms A and B are non-hygroscopic and do not deliquesce at RH up to 95%. According to results from TGA, which showed a negligible weight loss upon heating, both forms are ansolvates with little or no residual solvents.

An initial polymorphism screen was carried out in which slurry crystallization experiments of the neutral form (form B) in 12 different solvents at three different temperatures (5°C, 22°C, and 40°C) were performed. No additional crystal forms confirmed by XRPD (data not shown) were found after 2 weeks. Slurry experiments with mixtures of form A and form B were performed in order to find out which of the two forms was the energetically most stable. In all these slurry experiments, carried out in 2-propanol (5°C, 22°C, and 40°C) or isooctane (70°C), form A was found to convert into form B, which suggest that, in the investigated temperature range, form B is the most stable of the two forms and therefore also most suitable for further development.

Salt formation is one of the primary approaches used to modify the physical properties of a drug substance[1,15]. Here, in order to investigate alternatives to the free base, with the aim of finding a drug compound with optimal physicochemical properties (primarily higher melting point), a salt screen was performed. A total of 40 pharmaceutically acceptable acids were examined for their ability to form crystalline salts with the neutral form of AZD1305. The screen indicated formation of crystalline salt with two acids, forming the fumarate and the maleate salts of AZD1305.

Solid-state properties of the fumarate salt of AZD1305

Small quantities (about 50–100 mg) of three batches of the fumarate 1:1 salt were obtained and characterized. The characterized form was crystalline and showed a melting point onset of about 120°C, which is higher than the free form (about 90°C, see above). However, the material is hygroscopic and undergoes deliquescence at 95%RH (Table 1). Both of these properties are disadvantages and may lead to instability in the solid dosage forms and make the development challenging. An initial polymorphism screen was performed using the same equipment, materials, and approaches as for the free base, revealing a complicated pattern of different solvates, depending on which solvent was used. No further evaluation of the fumarate salt of AZD1305 was performed. The solvate formations, in combination with added weight from the counter-ion to the active compound, did not warrant any further investigation. Fumaric acid (and maleic acid, see below) has a molecular weight of 116 g/mol, which implies that in an 1:1 complex, the counter-ion increased the weight of the test compound by 27%. This is a disadvantage for an already high-dose compound. It is always more favorable to have one single component to follow (i.e. just the active compound) from a development perspective.

Solid-state properties of the maleate salt of AZD1305

A salt with maleic acid (1:1) was synthesized (about 100 mg). XRPD, DVS, and DSC data indicated a crystalline material that melts with an onset of about 90°C (Table 1). An initial polymorphism screen was performed using the same equipment, materials, and approaches as for the free base, revealing a complicated pattern of different solvates. The absence of an increase in melting point while increasing the molecular weight, as compared to the free base, made the maleate salt less attractive for further development.

Solid-state stability of the free base

The background to undertaking accelerated stability studies in the pharmaceutical industry, with elevated temperatures and/or sometimes moisture, is to be able to predict the shelf-life of the compound at room temperature. An increase in temperature will often produce an increase in decomposition (provided that the compound degrades at all). Often this behavior follows an Arrhenius

Table 1. Solid-state properties of AZD1305 neutral form, fumarate salt, and maleate salt.

Substance	Forms	DVS-water uptake at 80%RH (%, w/w)	DSC-thermal events on heating	TGA-weight loss (%, w/w)
AZD1305 (neutral form)	A	0.1%	Melting: T_m (onset) = 90°C; ΔH = 79 J/g	Less than 0.1% up to 110°C
AZD1305 (neutral form)	B (most stable)	0.04%	Melting: T_m (onset) = 90°C; ΔH = 89 J/g	Less than 0.2% up to 110°C
AZD1305 (fumarate salt)		7%, deliq. 95% RH	Melting: T_m (onset) ≈ 120°C	≈1.4% up to 110°C
AZD1305 (maleate salt)		1.7%	Melting: T_m (onset, double peak) ≈ 90°C	≈1.2% up to 110°C

DVS, dynamic vapor sorption; DSC, differential scanning calorimetry; ND, not determined; TGA, thermogravimetrical analysis.

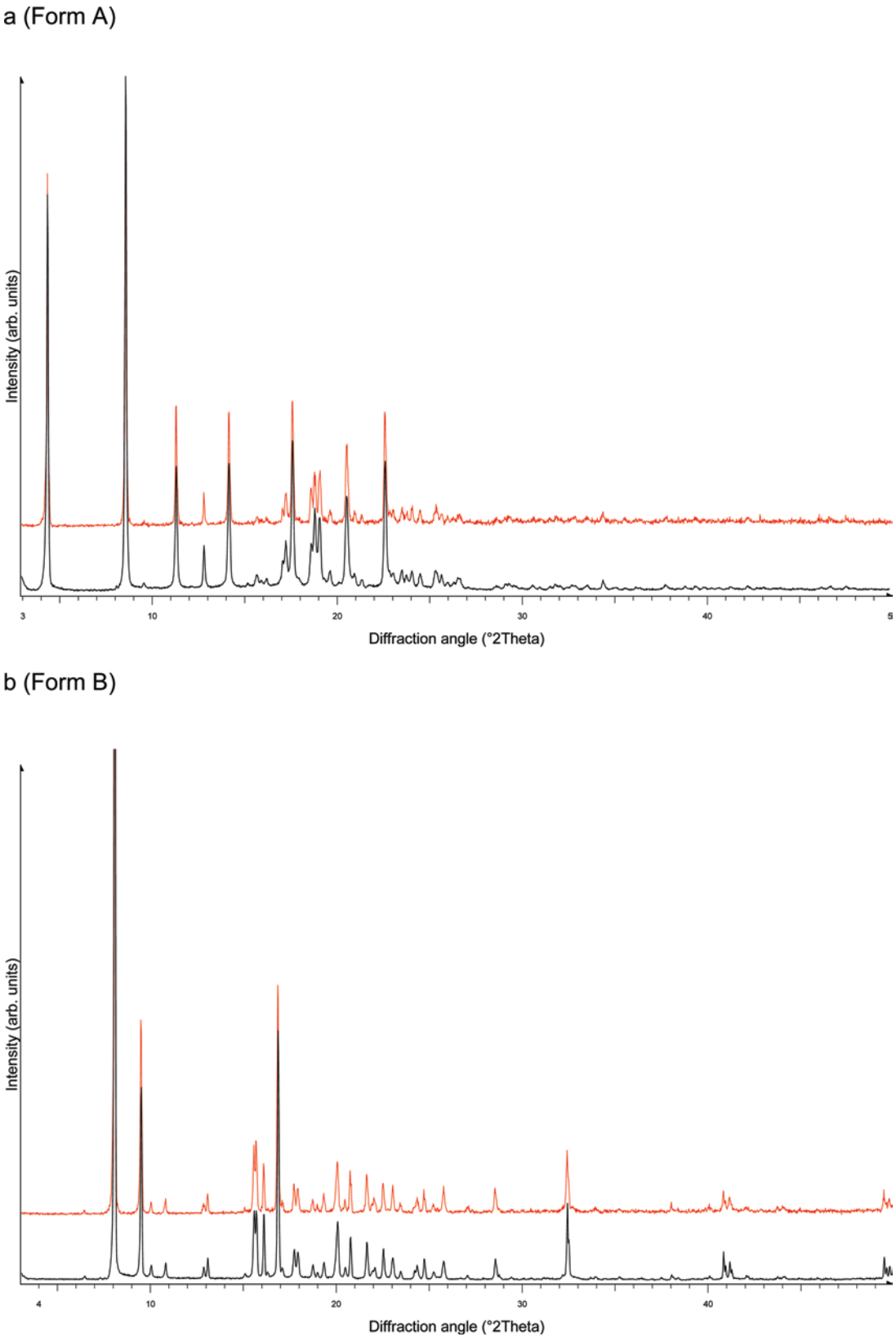

Figure 2. (A) Powder X-ray diffraction pattern of form A of AZD1305 as an original sample and the same sample stored for 5 years (the lower one) in a closed container, protected from light, at room temperature. (B) Powder X-ray diffraction pattern of form B of AZD1305 as an original sample and the same sample stored for 5 years (the lower one) in a closed container, protected from light, at room temperature.

relationship[12]. Evaluation of water uptake is included from a handling and packaging perspective. The presence of moisture may also lead to changes in form and degradation.

The chemical stability of AZD1305 (forms A and B) was investigated at five different conditions; –20°C, 25°C, 40°C/75%RH, 50°C, and 80°C. At –20°C, 25°C, 40°C/75%RH, and 50°C, no degradation could be observed (Tables 2 and 3). Bulk batches of forms A and B were stored at ambient conditions, at room temperature, in two closed vials. The samples were analyzed after 5 years. No degradation had occurred, in agreement with the observation of the sample stored at 50°C and the X-ray data shown below.

After 1 week at 80°C, the compound (forms A and B) showed a tendency to degradation, which was obvious after 1 month. Five peaks (0.20–0.67%) were formed. After 3 months the compound (forms A and B) has melted and no measurements were performed (Tables 2 and 3). The degradation pathway often changes with elevated temperature, i.e. there are deviations from the Arrhenius plot at certain temperature levels. For the present compound, this occurs at ≥50°C. Thus the observed events at 80°C were not surprising, since it is close to the melting point of the compound. The condition of the 5-year samples further supported that the degradations taking place at 80°C are not relevant for the lower temperatures, i.e. different degradation routes occur for the compound in solid state, depending on different environmental conditions. In summary, the 5-year stability data verifies the conclusion drawn from the stress tests (≤50°C), i.e. no critical degradation occurs at ambient conditions during the time period investigated. No Arrhenius plot was performed at temperatures ≤50°C due to lack of degradation. At 80°C, forced degradation pathways were initiated, which were not relevant at lower temperatures.

Information on transitions at elevated temperature, with or without presence of elevated moisture, can give clues about possible issues in long-term stabilities and can provide guidance on drying steps. XRPD measurements were performed on the samples stored at 40°C/75%RH and 50°C after about 4 months. No changes in the solid state were observed in form B. A tendency to formation of form B was observed in samples of form A at

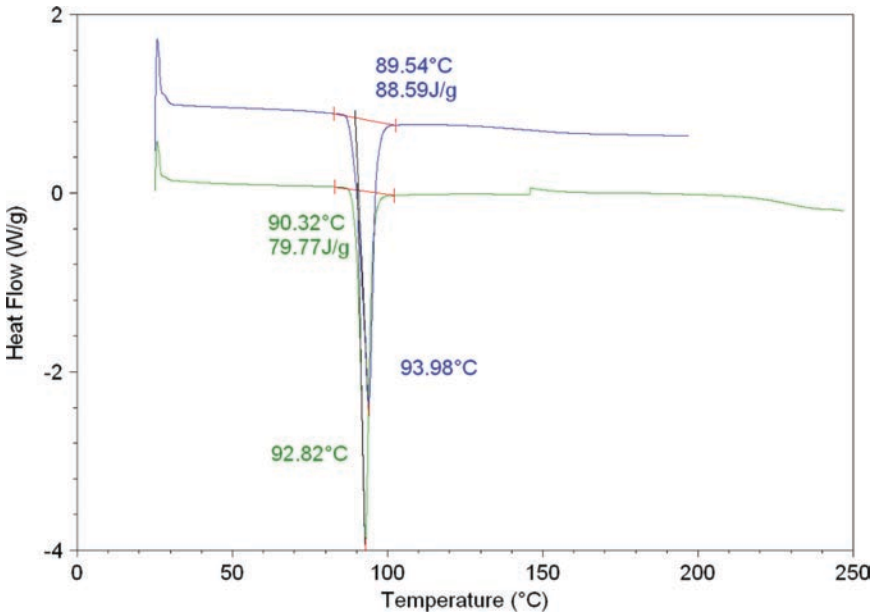

Figure 3. The differential scanning calorimetry profiles of form A (lower) and form B (upper) of AZD1305 showed one endothermic peak at about 90°C (onset) for both forms due to the melting of respective form.

Table 2. Solid-state chemical stability of AZD1305 (form A) at 25°C, 40°C/75%RH, 50°C ambient humidity, and 80°C ambient humidity.

		Purity (%)			
Conditions	Initial value	1 week	1 month	3 months	4 months
25°C (closed container)	100.0	100.0	100.0	100.0	ND
40°C/75% RH (open container)	100.0	100.0	100.0	100.0	ND
50°C ambient humidity (closed container)	100.0	100.0	100.0	100.0	ND
80°C ambient humidity (closed container)	100.0	99.8	97.6	ND[a]	ND
–20°C (closed container)	100.0	ND	ND	ND	100.0

ND, not determined.
[a]The compound has melted.

Table 3. Solid-state chemical stability of AZD1305 (form B) at 25°C, 40°C/75%RH, 50°C ambient humidity, and 80°C ambient humidity.

		Purity (%)			
Conditions	Initial Value	1 week	1 month	2.5 months	3 months
25°C (closed container)	99.9	ND	ND	100.0	ND
40°C/75%RH (open container)	99.9	ND	ND	99.9	ND
50°C ambient humidity (closed container)	99.9	ND	ND	99.9	99.9
80°C ambient humidity (closed container)	99.9	99.7	97.7	ND[a]	ND[b]
–20°C (closed container)	99.9	ND	99.9	ND	99.9

ND, not determined.[a]The sample was not inspected. [b]The compound has melted.

40°C/75%RH (further supporting form B to be the most thermodynamically stable form) but not at 50°C. Both bulk batches of forms A and B were stored at ambient conditions, in closed containers, at room temperature for 5 years (Figure 2). No changes in crystal form were observed, indicating that the tendency to form transition of the compound (see above) was related to the presence of elevated moisture. There does not appear to be any stability-related issues for the solid state of the free base during ambient conditions, using form B as the form for development. This combined information suggests that AZD1305 will likely be stable, as free base, under normal processing (however, with a lower and more controlled drying temperature than normal, see below) and storage conditions, which helps reduce future risks during development for launch. Moreover, both crystal forms were unaffected chemically when exposed to light.

Particle size of AZD1305

SEM graphs of AZD1305, forms A and B are presented in Figure 4. The particle size distribution of both batches was broad (2–200 μm), according to ocular evaluation of the micrographs, which, together with the needle morphology, caused problems in flowability.

AZD1305 was crystallized from isopropylether (IPE), which created problems associated with a bad odor due to the residual of IPE, in spite of the low amounts (0.05–0.08%) present. Other solvents were tested as crystallization media but were found to be inferior alternatives to IPE. However, it was observed that the sensation of odor could be reduced by increasing the particle size of the substance. When larger particles were produced the odor was largely suppressed, or even abolished. Because there are no solubility-related issues for the compound, a moderate increase in particle size is acceptable. An increase in particle size decreases the surface exposed to the surrounding environment, which also reduces the amount of IPE exposed to the air leading to decreased, or even abolished, odor. In addition, when AZD1305 was wet-milled according to earlier described conditions[35,36], the odor increased.

The low melting point makes milling and micronization of AZD1305 challenging at ambient temperatures. However, due to the favorable solubility, these process steps are probably not necessary. Furthermore, the low

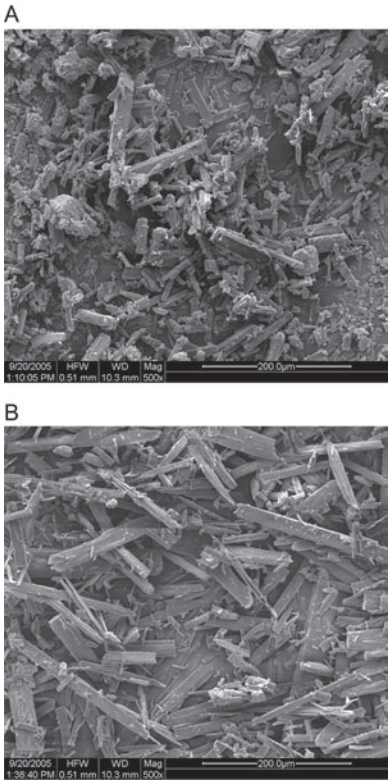

Figure 4. Scanning electron microscopy imaging of AZD1305, (A) form A and (B) form B.

melting point of AZD1305, 90°C, was the reason behind the observed stickiness of tablet masses in compaction simulation studies (AstraZeneca, data on file). These, together with the relatively large daily dose for an ER formulation, of 250–500 mg, are critical issues in successful formulation work of solid dosage forms.

Small-scale wet granulation

It is also important to investigate the wet granulation possibilities and evaluate the solid state after adding ethanol or water, the latter being more preferable from environmental aspects. Conventional drying conditions should also be investigated and evaluated, because it is an important step in future large-scale synthesis. In Figure 5, XRPD graphs are presented. These show that there was no change in crystal form, which is consistent with solid-state results for form B. However, from ocular

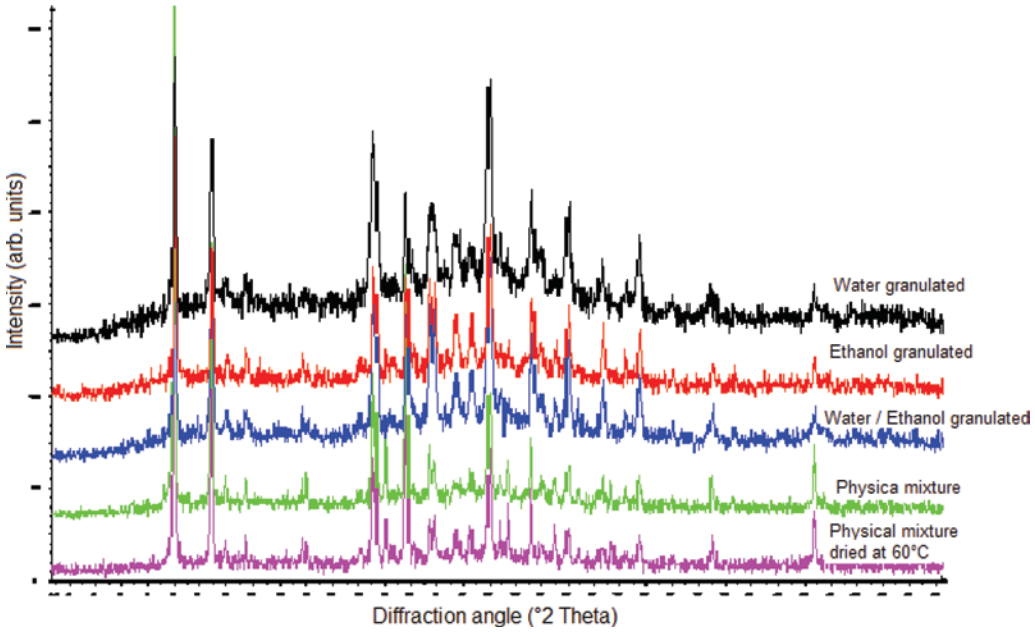

Figure 5. Powder X-ray diffraction pattern of form B of AZD1305 after granulation experiments and physical mixtures.

evaluation, the crystallinity of AZD1305 was decreased by wet granulation (Figure 5). Drying at 60°C has no effect on crystal form, which is in line with results from solid-state stability tests in 40°C/75RH and 50°C (see above). Based on these and solid-state results, it can be concluded that wet granulation of this drug substance is feasible although it must be considered that the unavoidable increase in amorphous content might have a lowering effect on stability.

pK_a

The ionization properties were analyzed using CE and AZD1305 was found to have one basic function with a pK_a = 9.9 ± 0.1. However, as a first step before experimental measurements, a prediction approach was performed, using ACD[14] to estimate the pK_a of the compound. This approach resulted in two alkaline pK_a values of 7.1 and 4.0, attributed to the two nitrogen molecules in the oxabispidine group of the molecules (Figure 6). The discrepancy between observed and calculated pK_a values might indicate that the protonization occurs in the nitrogen at the secondary amine group (outside of the oxabispidine group, see Figure 1). Further support for such a behavior is given by the observed degradation pattern discussed below.

Solubility

The solubility of AZD1305 (forms A and B) was determined in aqueous solution at pH 7–13) after 1 day of equilibration (Figure 7). There are some important considerations to be aware of when discussing solubility. The first is to confirm whether the solubility is at equilibrium or whether it should be considered as apparent (kinetic) solubility. To confirm the former, a number of analyses were performed to find out if and when the solubility reached equilibrium, i.e. when there were no changes in solubility with time. For the present compound, equilibrium was reached before

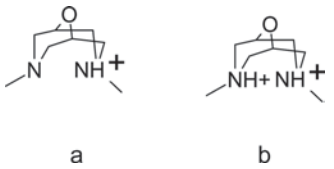

Figure 6. Possible basic moieties of AZD1305.

24 h. Because there always is a risk for possible salt formation with the buffer components, NaOH/HCl were used to adjust pH. The solubility is pH dependent with solubility increasing at more acidic pH (Figure 7, Tables 4 and 5). The results confirmed the pK_a values measured earlier (see above). To verify that there were no ionic effects on the solubility, pure water and a phosphate-buffered solution were used to adjust some pHs that correlate well with the curve in Figure 7 (Table 5). The intrinsic solubilities of the two polymorphs of AZD1305 were found to be essentially the same at around 0.1 mg/mL. The precision in the solubility measurements does not permit one to conclude which form is thermodynamically more stable. However, form B was found to be somewhat more stable as indicated from observations on conversion from form A to B in slurry experiments and in solid-state stability studies (see above). The slightly higher melting enthalpy of form B is in line with these findings. As the solubility is so high at lower pH for the free base of the substance, the compound will significantly elevate the pH in high concentration solutions significantly (Tables 4 and 5) due to an inherent buffer capacity of the compound itself. Even in 0.1 M HCl, the solution obtained a neutral pH. This is a positive property of the compound that contradicts the degradation at low pH (see below).

Another consideration (see above) is whether a change in crystal form has occurred during the solubility experiments. Hence, the remaining solid phases from

the solubility experiments with AZD1305 were analyzed by XRPD. In all cases, the remaining solid phases were identical with the original form, in agreement with the findings from the slurry experiments (see above).

Stability in solution

The stability in solution was investigated under 12 different conditions, including variation of pH, temperature, and absence or presence of light (Table 6). Obviously, pH and temperature affect the stability of the compound in solutions. At low pH, the degradation was fast and similar in light and darkness. Figure 8 shows the degradation profiles at pH 3, 6, and 8.1 after the initial measurement and day 15. Just one polar (in the front) degradation product was observed (see Figures 8 and 9 and below). Also at pH 1, just one (the same) degradation product appeared. However, the degradation rate increased significantly. After 15 days, there was no AZD1305 left. The

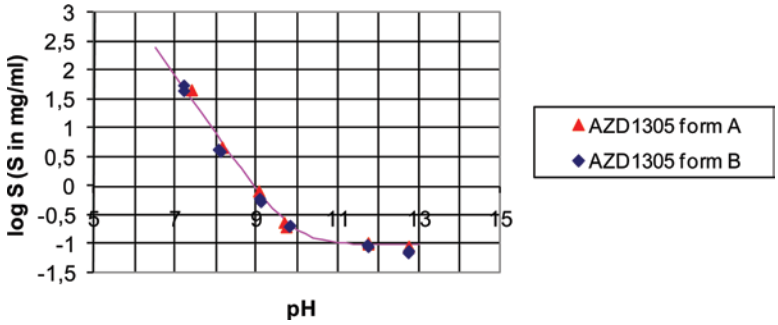

Figure 7. Solubility vs. pH of AZD1305 after one day of equilibration. The solid line represents the Henderson–Hasselbach equation, with the fit parameters pK_a 9.9 and an intrinsic solubility of about 0.1 mg/mL.

Table 4. Solubility in aqueous solutions of AZD1305 (form A) after 24 h of equilibration at room temperature.

Solutions/ionic strength[a]	pH[b]	Concentration (mg/mL)
0.1 M NaOH	12.7	0.09
0.01 M NaOH/I 0.1	11.8	0.1
0.001 M HCl/I 0.1	9.1	0.8
0.01 M HCl/I 0.1	8.1	5
0.1 M HCl/I 0.1/1	7.4	40
Water[c]	9.7	0.2

[a]Where applicable, NaCl was used to increase ionic strength to 0.1 M (not in H_2O).
[b]Measured pH value.
[c]To verify that there were no ionic effects on the solubility.

Table 5. Solubility in aqueous solutions of AZD1305 (form B) after 24 h of equilibration at room temperature.

Solutions/ionic strength[a]	pH[b]	Concentration (mg/mL)
0.1 M NaOH	12.7	0.07
0.01 M NaOH/I 0.1	11.7	0.09
0.001 M HCl/I 0.1	9.1	0.6
0.01 M HCl/I 0.1	8.1	4
0.1 M HCl/I 0.1/1	7.2	50
Phosphate buffer pH 6.8/I 0.1	7.7	9.3
Water[c]	9.8	0.2

[a]Where applicable, NaCl was used to increase ionic strength to 0.1 M (not in H_2O).
[b]Measured pH value.
[c]To verify that there were no ionic effects on the solubility.

Table 6. Stability of AZD1305 (0.3 mg/mL, form A) in phosphate buffer solutions, ion strength 0.1 M, and water.

		Purity (%)			
Conditions	Initial value	Day 1	Day 2	Day 7	Day 15
RT (light)					
pH 1.0	98.1	41.6	ND	ND	ND
pH 8.1	100	99.9	99.8	99.5	98.6
RT (dark)					
pH 1.0	98.1	40.1	ND	ND	ND
pH 3.0	99.9	98.8	97.8	93.4	86.3
pH 6.0	100	99.9	99.8	99.3	98.6
pH 8.1	100	99.9	99.8	99.4	98.7
Water	100	99.9	99.7	99.3	98.7
50°C (dark)					
pH 1.0	98.1	0.0	ND	ND	ND
pH 3.0	99.9	64.0	41.8	6.2	ND
pH 6.0	100	92.8	86.2	61.9	ND
pH 8.1	100	93.3	86.6	63.7	ND
Water	100	94.6	89.1	66.2	ND

ND, not determined; RT, room temperature.
pH in water solutions was measured to 8.3.

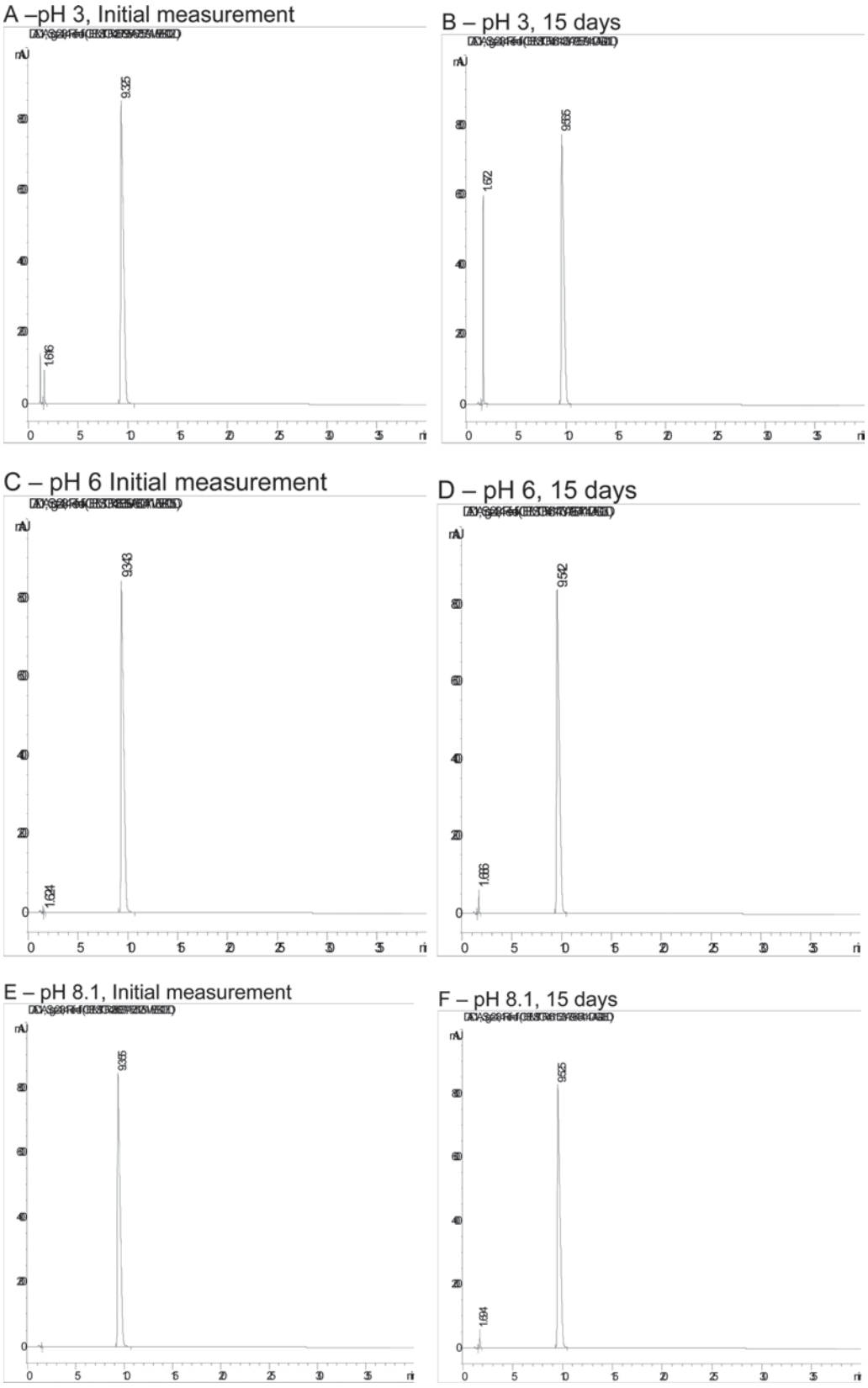

Figure 8. Chromatograms of AZD1305; initial and 15-day analyses at (A, B) pH 3, (C, D) pH 6, and (E, F) pH 8.1 showing the appearance of AZD1305amine (see Figure 9) after about 1.6 min.

differences observed in the figures for day 1, at pH 1, in light and darkness (Table 6), are due to the exact time-point when the actual analysis was performed. At low pH, some degradation product could have been formed rapidly before the initial measurement was performed, during storage of the bulk or formed as an impurity from the API synthesis. At high temperature, the degradation increases further. Further investigation was focused on low pH, conditions relevant in the gastric tract (pH 1–3) and for the liquid formulation (pH about 3) (see below).

To further evaluate the degradation at gastric pH, additional measurements were performed at two different concentrations, 0.3 mg/mL and 0.03 mg/mL at pH 1.1. At pH 1.1 (35°C), the half-life was 110 min. This means that after 30 min, a realistic residence time in the gastric tract during normal, fasting conditions[37,38], 17% of AZD1305 had degraded. According to previous runs, the logarithm of the rate constant is directly proportional to pH in this pH range. The calculated half-life at pH 2.1 (35°C) is 1100 min (18 h). This corresponds to 2% degradation after 30 min. The "BOC"-group is cleaved off (the right hand side of the molecule, see Figure 1) in the gastric tract, a reaction that produces gas (CO_2), *tert*-butanol and the product molecule, AZD1305amine (Figure 9). The initial prediction of the degradation route was verified with MS. The degradation product was synthesized (verified with NMR) and spiked in an HPLC run. The possible side-effect profile of the degradation product must be evaluated carefully, but the major effect is the loss of the active compound. However, there are a number of factors that counteract the degradation of AZD1305 *in vivo* by elevating the gastric pH. For instance, the compound itself elevates the pH (see above). In addition, the intended treatment, i.e. an ER formulation, will protect the actual drug from the gastric juice and release the compound mainly in colon, where the compound has good permeability and stability (see Introduction). Moreover, taking the drug with food results in a higher gastric pH[39,40].

Solubility in vehicles

There is a strong pH dependency for the solubility of AZD1305. Using equimolar amounts of tartaric acid dissolved in 9 mg/mL sodium chloride, the solubility was >100 mg/mL of AZD1305 (pH 3–4). Tartaric acid was used to lower the pH, as it acts as a buffer, giving a final pH around pH 3–4. The intention was to avoid lower pH, as the compound degrades (see above) but reaches enough solubility in the liquid stock formulations for the *in vivo* studies. Sodium chloride was included as a tonicity modifier to make it possible to use the same formulation for per oral and i.v. administration. Also mannitol, glucose, glycerol, and sorbitol were good choices to minimize the risk of hemolysis and vessel damage during parenteral administration.

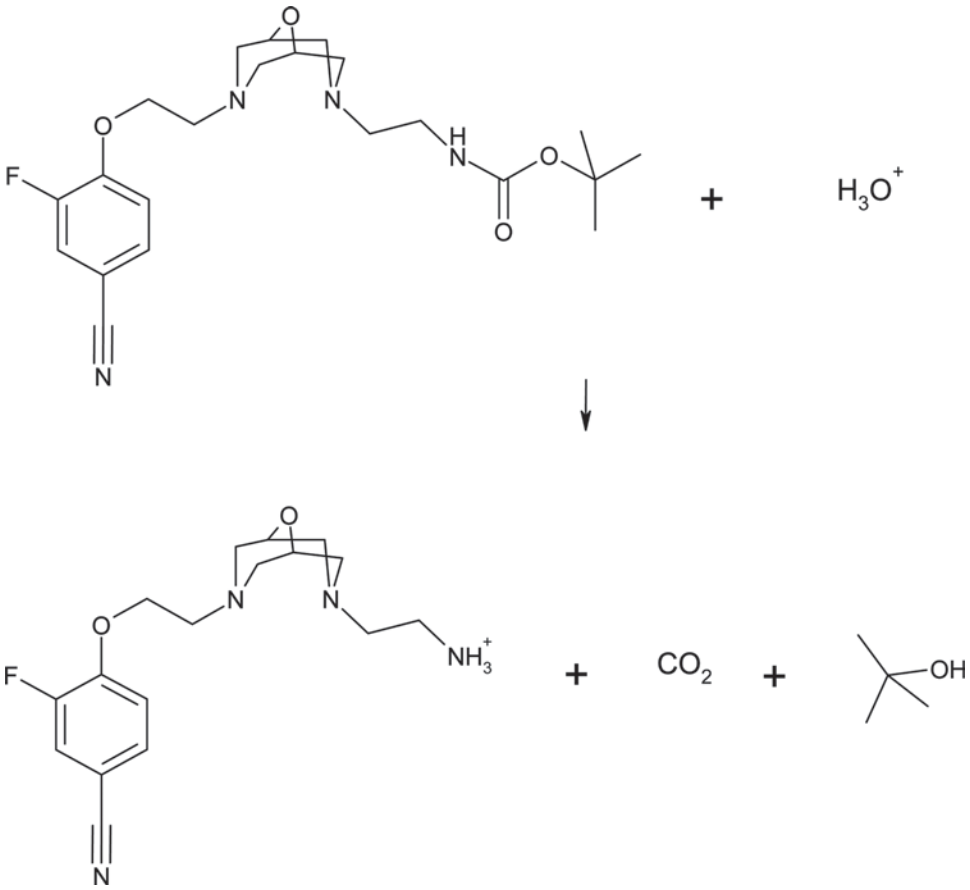

Figure 9. The proposed degradation route of AZD1305 in solution at the physiological pH interval.

Conclusion

The neutral form of AZD1305 seemed to be the most suitable agent for product development even though it has a fairly low melting point (approximately 90°C) and occurred as two different crystal forms. Form B was the most stable thermodynamically in the temperature interval of interest. Even though the melting point of the fumarate salt is significantly higher (approximately 120°C), its hygroscopic properties together with the existence of other crystal forms (solvates) render it less suitable for product development as compared to the free base. The maleate salt of the compound had no advantages compared to the neutral form of AZD1305. AZD1305 undergoes a significant degradation at low pH but has an intrinsic counteracting property (i.e. strong buffering capacity), which together with the similar counteracting effect of the ER formulation reduces, or even eliminates, the risk of drug absorption variability. AZD1305 was crystallized from IPE. This approach created problems with a bad odor at low levels (0.05–0.08%) of residual IPE. Increasing the particle size of the substance reduced the odor. As the solubility at physiological conditions is favorable, a moderate increase in particle size was acceptable.

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Declaration of interest

All authors are employees of AstraZeneca. The authors report no conflicts of interest.

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