Organic Process Research & Development **2007**, *11*, 365–377

An Approach to Early-Phase Salt Selection: Application to NBI-75043

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Abstract:

A strategy for the salt selection of NBI-75043 is presented and summarized in a decision-tree format. This is followed by the first scale-up of the process, confirmation of a new polymorph, and description of a method for its conversion to the initial form.

Introduction

Four types of histamine receptors have been characterized in humans (H1−H4).[1] The H1 receptor is the primary receptor involved in allergic rhinitis symptoms and motion sickness. H1 Antagonists such as diphenhydramine hydrochloride block histamine binding in peripheral tissues, thereby reducing symptoms of allergic rhinitis.[2] One side effect of diphenhydramine results from its penetration of the blood−brain barrier, resulting in CNS depression. Accordingly, H1 antagonists with good blood−brain barrier penetration are thought of as likely candidates for next-generation sleep therapies.[3] NBI-75043 is a highly selective, highly potent, H1 antagonist that was under evaluation for safety and efficacy in the treatment of insomnia.

NBI-75043 is a small molecule (MW = 314) that was synthetically prepared and initially isolated from a racemic mixture through classical resolution with tartaric acid. The desired enantiomer was ultimately isolated as a crystalline L-tartrate salt with good purity (95−97% AUC) and solubility (>250 mg/mL) in water. Later route development work resulted in an enantioselective synthesis that eliminated the need for resolution of a racemic mixture.[4]

The initial lots of the tartrate salt produced were dried to a constant weight, but were found to be ethanol solvates (∼6% w/w; 9% theoretical for 1 NBI-75043:1 L-tartaric acid:1 ethanol), as determined by C, H, and N and GC residual solvent analyses. Additionally, the tartrate salt was found to be a low-melting (∼94 °C) hygroscopic solid. Furthermore, the ethanol solvate was found to readily exchange ethanol for water within one week under accelerated stability conditions (40 °C/75% relative humidity).

Owing to these inherent negative properties for an API, this salt form of NBI-75043 was recognized as being unacceptable for the long-term studies of this development drug candidate. An approach towards salt selection for NBI-75043 and drug candidates in general is presented here.

Salt Selection Strategy

Our goal was to perform an efficient but at the same time thorough selection process that would allow us to choose a salt that is free of the problems such as the ones associated with the tartrate salt of NBI-75043. Several excellent articles and books have been published on the general subject of salt selection.[5] Surprisingly, a detailed decision tree or workflow diagram for such an effort was not readily available in the literature. Thus, a straightforward approach for this effort was developed and a decision tree (Figure 1) was drafted that could be readily utilized by Process R&D scientists. As is typical in early-stage development, quantities of NBI-75043 were limited. However, there were ample amounts of its unused enantiomer, NBI-75042, available from resolution. Thus, NBI-75042 was used for screening achiral counterions, and those that showed promise were duplicated and scaled up using NBI-75043.

NBI-75043 free base is a crystalline material with a melting point (64 °C) that is lower compared to that of the tartrate salt. To prepare a form with a higher melting point more suitable for formulations, only salts of NBI-75043 were considered in the selection process (vide infra). Our criteria for acid selection was to employ acids that are generally regarded as safe (GRAS) by the FDA and have previously been used in FDA-approved marketed drugs.[5a] Additionally, the acids would need to be of sufficient strength to protonate

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[1] (a) Leurs, R.; Smit, M. J.; Timmerman, H. *Pharmacol. Ther*. **1995**, *66*, 413−463. (b) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erland, M. G. *Mol. Pharmacol*. **1999**, *55*, 1101−1107. (c) Schneider, E.; Rolli-Derkinderen, M.; Arok, M.; Dy, M. *Trends Immunol*. **2002**, *23*, 255−263.

[2] Huang, Z. L.; Qu, W. M.; Li, W. D. Mochizuki, T.; Eguchi, N.; Wantanabe, T.; Urade, Y.; Hayaishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9965−9970.

[3] (a) Kaneko, Y.; Shimada, K.; Saitou, K.; Sugimoto, Y.; Kamei, C. *Methods Find. Exp. Clin. Pharmacol.* **2000**, *22*, 163−168. (b) Nicholson, A. N.; Pascoe, P. A.; Turner, C.; Ganellin, C. R.; Greengrass, P. M.; Casy, A. F.; Mercer, A. D. *Br. J. Pharmacol.* **1991**, *104*, 270−276. (c) Timmerman, H. In *Therapeutic Index of Antihistamines*; Church, M. K., Rihoux, J.-P., Eds.; Bern, Hogrefe & Huber: New York, Toronto, 1992; pp 19−31.

[4] Details of route development and scale-up work will be the subject of a future paper.

[5] (a) Gould, P. L. *Int. J. Pharm.* **1986**, *33*, 201−217. (b) Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. *J. Pharm. Sci.* **1977**, *66* (1), 1−19. (c) Maurin, M. B.; Rowe, S. M.; Koval, C. A.; Hussain, M. A. *J. Pharm. Sci*. **1994**, *83* (10), 1418−1420. (d) Bastin, R. J.; Bowker, M. J.; Slater, B. *Org. Proc. Res. Dev.* **2000**, *4*, 427−435. (e) *Handbook of Pharmaceutical Salts Properties, Selection, and Use*; Stahl, P. H., Wermuth, C. G., Eds.; Wiley-VCH: Weinheim, 2002.

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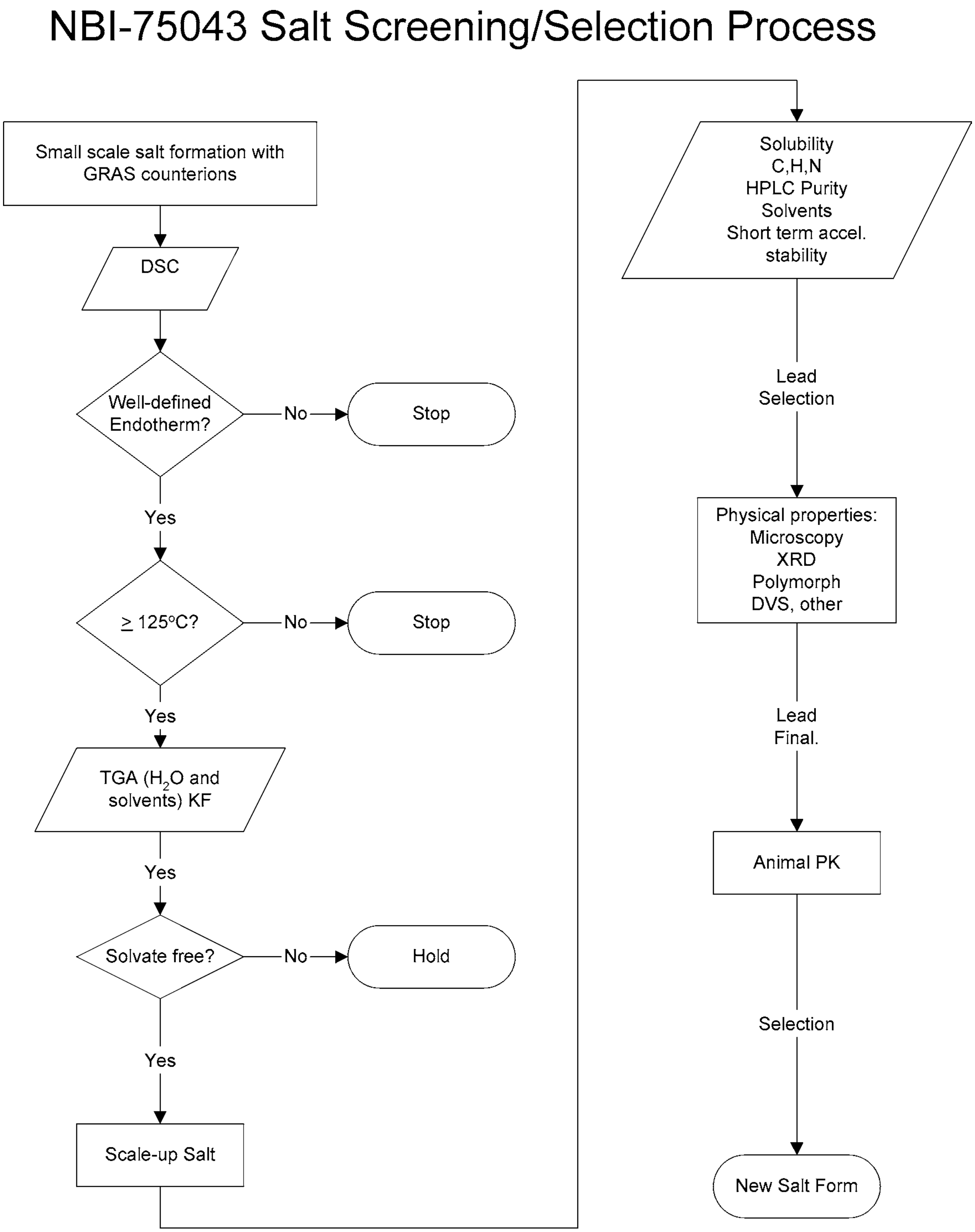

Figure 1. **Salt selection decision tree.**

NBI-75043 (pK_a = 8.75).[6] Initially, 14 acids were selected: acetic acid (HOAc), benzoic acid, benzenesulfonic acid (BSA), citric acid, fumaric acid, hydrobromic acid (HBr), hydrochloric acid (HCl), maleic acid, methanesulfonic acid (MsOH), nitric acid (HNO_3), phosphoric acid (H_3PO_4), salicylic acid, sulfuric acid (H_2SO_4), and *p*-toluenesulfonic (tosic) acid.[7] If none of the salts formed with these acids passed our selection criteria, another set of acids (that were viewed as second tier) were to be selected and screened. One molar equivalent of the selected acids was added to an ethanolic solution of NBI-75042, and the solutions were concentrated in vacuo in order to azeotrope off any water that was present. HOAc, HBr, and H_2SO_4 were added as concentrated aqueous solutions, and tosic acid was added as a hydrate; therefore, this additional drying step was used to ensure that salts of these acids were not dissolved in residual water and inadvertently discarded. The concentrated residue was screened for solubility in 10 different solvents (Table 1) covering a wide range of polarity. The solubility

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(6) To form a stable salt, it is recommended to have 3 p*K* units difference between the base and counterion (see ref 5d).

(7) Caution should be exercised when selecting maleic or fumaric acid as these acids are known to form covalent adducts with amines. See Anderson, N. G. *Practical Process Research & Development*; Academic Press: San Diego; 2000; pp 239−240.

Table 1. **Solvent polarity**[a]

solvent	E_T
water (H_2O)	63.1
methanol (MeOH)	55.5
ethanol (EtOH)	51.9
isopropanol (IPA)	48.6
acetonitrile (ACN)	46.0
acetone	42.2
tetrahydrofuran (THF)	37.4
ethyl acetate (EtOAc)	N/A
methyl *tert*-butyl ether (MTBE)	N/A
hexanes	33.1

[a] Data from March, J. *Advanced Organic Chemistry*, 3rd ed.; John Wiley & Sons: New York, 1985; p 319.

screen was performed in two ways. First, the solubility of the salts in single solvents at both hot and cold temperatures was evaluated. Samples were heated, solubility was noted, and then samples were allowed to cool without control to ambient temperatures where the solubility was again noted. Second, if salts did not yield a solid from a single solvent, antisolvents were used to force solid formation or oiling of the salt. Antisolvents were determined from the solubility screen.[8] This procedure was repeated for each of the acid salts made. The solubility screen was run at a scale sufficient to provide material for isolation by vacuum filtration.

Once solids were isolated, our first screening tool for assessing the nature of the salts was differential scanning calorimetry (DSC) analysis. DSC was selected as our initial screening tool primarily due to the convenience and the nature of the information it provides. Melting points, crystallinity, solvates, and/or presence of polymorphs may be all be determined from a single piece of equipment as evidenced by multiple endotherms, poorly defined endotherms, and endotherms near the boiling point of solvents.[9] Additionally, through the use of a sampling robot, the efficiency of DSC measurements was acceptably high. Evidence of crystallinity, presented as a well-defined endotherm, was set as a prerequisite. Additionally, we set a melting point of $\geq$125 °C to avoid incompatibilities with the mechanical forces typically encountered during the manufacture of drug product such as compaction or heat of mixing as a function of shear force.[10] A higher-melting salt would also be less likely to require specialized shipping and storage conditions (i.e., refrigeration) and was more likely to be stable for a longer period of time as compared with a lower-melting salt. We also decided to hold off choosing salts that showed evidence of polymorphs for a more thorough solvent screen (to be evaluated if necessary).

Salts that proceeded past the DSC analysis were next evaluated by thermogravimetric analysis (TGA) and Karl Fischer (KF) titration. Salts that appeared to contain solvates were set aside.

Next, the aqueous solubility of these salts was determined. The tartrate salt had excellent solubility, possibly enhanced due to the fact that it was an ethanol solvate. Consequently, it was expected that we would not choose a salt that is poorly soluble which would complicate both nonclinical and clinical studies. As such, we set a minimum solubility of 1 mg/mL in water.

Simultaneously, these salts were analyzed for purity by HPLC area under the curve (AUC), with our criterion for further progression through the decision tree being no loss in purity. Salts that had an increased level of purity were ranked higher.[11] The samples were also analyzed for residual solvents to determine whether solvates were present or if the salts were difficult to dry, indicating potential processing challenges as scale was increased during manufacturing.

Following this, the physical properties of the salts were further investigated. The salts were subjected to combustion analysis (C, H, and N) as both a rough measure of purity and to confirm the salt stoichiometry. Alternate methods such as ion chromatography and HPLC for determining salt stoichiometry were considered. However, as one of our goals at the outset was to minimize the labor required to perform this screen, we opted to hold off on the development of new methods until it became necessary.

The isolated salts were observed by optical microscopy under 10× magnification to determine their bulk morphology (needles, plates, etc.) and for an early screen for polymorphs. The potential for polymorphs was further examined by powder X-ray diffraction (PXRD) analysis.

Additionally, the salts were subjected to DVS (dynamic vapor sorption) in order to gauge their hygroscopicity. The final phase of our physical attributes analysis was to determine how these chosen salts would behave in a physiological system. We had set a solubility criterion; however, we were still concerned that, in changing salts, we may significantly alter the bioavailability and therefore the exposure of our compound in vivo. To gain insight into how the new salts would behave in this respect, we took our top candidates and performed an animal pharmacokinetic (PK) study. Our desire was to have equivalent or better exposure with the new salt as compared to that with the tartrate salt of NBI-75043.

Results and Discussion

The results of our initial screen are shown in Table 2 and Figures 2−5.[12] From these results, further work on the tosylate salt was discontinued both because of its hygroscopicity and indications of multiple polymorphs. The maleate salt was deprioritized due to its high solubility in a variety of solvents. After eliminating these salts from contention, we had several promising leads. Eventually, three salt candidates of NBI-75043 (besylate, fumarate, HBr) were chosen to progress to a monkey pharmacokinetic (PK) study along with the tartrate as a direct comparator. The study was

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(8) Chen, C.-K.; Singh, A. K. *Org. Process Res. Dev.* **2001**, *5*, 509.

(9) (a) *Physical Characterization of Pharmaceutical Solids*; Brittain, H. G., Ed.; Drugs and the Pharmaceutical Sciences, Vol. 70; New York: Marcel Dekker, 1995; pp 14−18. (b) *Polymorphism in Pharmaceutical Solids*; Brittain, H. G., Ed.; Drugs and the Pharmaceutical Sciences, Vol. 95; New York: Marcel Dekker, 1999; pp 251−256.

(10) Rankell, A.; Lieberman, H. A. In *The Theory and Practice of Industrial Pharmacy*; Lachman, L., Lieberman, H. A., Kanig, J. L., Eds.; Lea & Febiger: Philadelphia, 1970; pp 62−67.

(11) Purity values reported exclude any contributions from the acids (if any) at 260 nm.

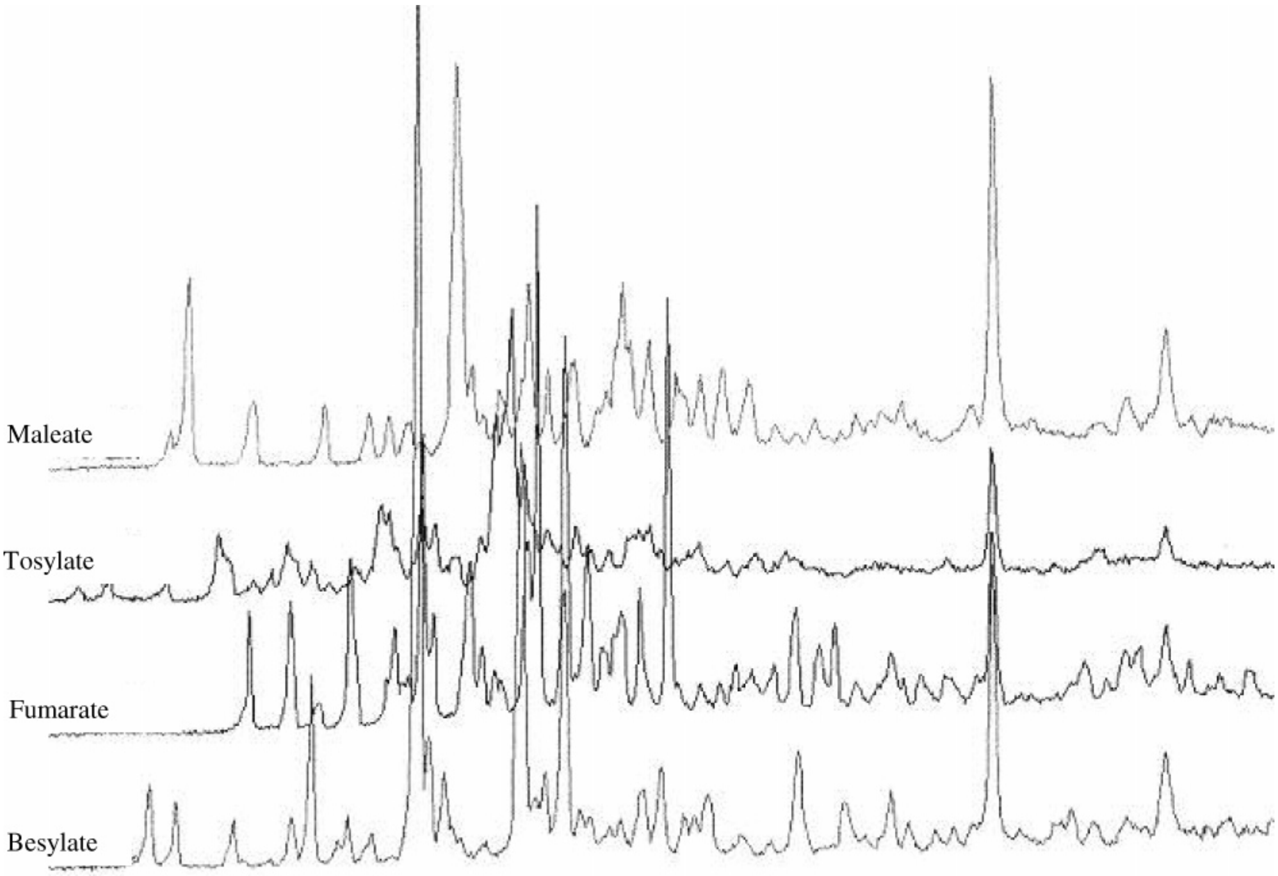

Figure 2. **PXRD of various salt forms.**

Table 2. **Initial salt analysis**

salt form (solvent)	besylate (ACN)	besylate (acetone)	besylate (EtOAc)	fumarate (IPA)	maleate (EtOAc)	tosylate (EtOAc)	HBr (EtOAc)
DSC	168.1 °C	167.5 °C	165.3 °C	167.0 °C	133.1 °C	142.4 °C	151.0 °C
residual solvent level	836 ppm	191 ppm	635 ppm	2370 ppm	3350 ppm	453 ppm	325 ppm
(ICH limit ppm)	(420)	(5000)	(5000)	(5000)	(5000)	(5000)	(5000)
moisture content w/w%	0.09	0.09	0.07	0.25	0.05	0.14	0.1
%LOD (to 150 °C)	0.01	0.10	0.01	0.30	0.07	0.10	0.71
moisture uptake w/w% @ 90% RH	<0.1%	<0.1%	<0.1%	≤ 1%	≤ 2.5%	≤ 12%	≤ 5%
purity (AUC)	96.5	96.6	96.0	93.3	95.0	96.0	99.9
PXRD	crystalline Form 1	crystalline Form 1	crystalline Form 1	single form	single form	multiple forms?	crystalline
physical appearance	needles	needles	needles	plates	plates	plates/needles	plates
recovery	79%	61%	84%	69%	19%	74%	50%

performed with an *N* of five non-naïve male cynomolgus monkeys dosed by oral gavage (PO) in four phases with a 1-week washout between doses. Blood samples were obtained at selected times starting from predose to 48-h post dose (Figure 6). With approximately the same PK as the tartrate salt and no clear pharmacokinetic differentiation between any of the salts tested, the besylate salt was selected for future development for the following reason. The besylate salt appeared to be the most desirable compared to the fumarate or HBr as it crystallized in high purity and yield from multiple solvents and in a single polymorphic form even when using BSA from several different vendors. This choice presented a challenge in that benzene is a potential impurity in benzenesulfonic acid and is an ICH class 1 solvent with a suggested limit of 2 ppm.[13] Because the salt formation would take place at the last step of the synthesis and little was known at this stage regarding the fate and purging of any benzene (introduced via BSA) from the final product, we set an expectation to control benzene to 2 ppm in the final product. Benzene was tested as a limit test of 2 ppm using a GC−MS method operating in single-ion monitoring (SIM) mode. Limits of quantitation for the method were established at the time of testing using a practical limit of quantitation (PLOQ) standard of 0.2 ppm. Benzene recovery

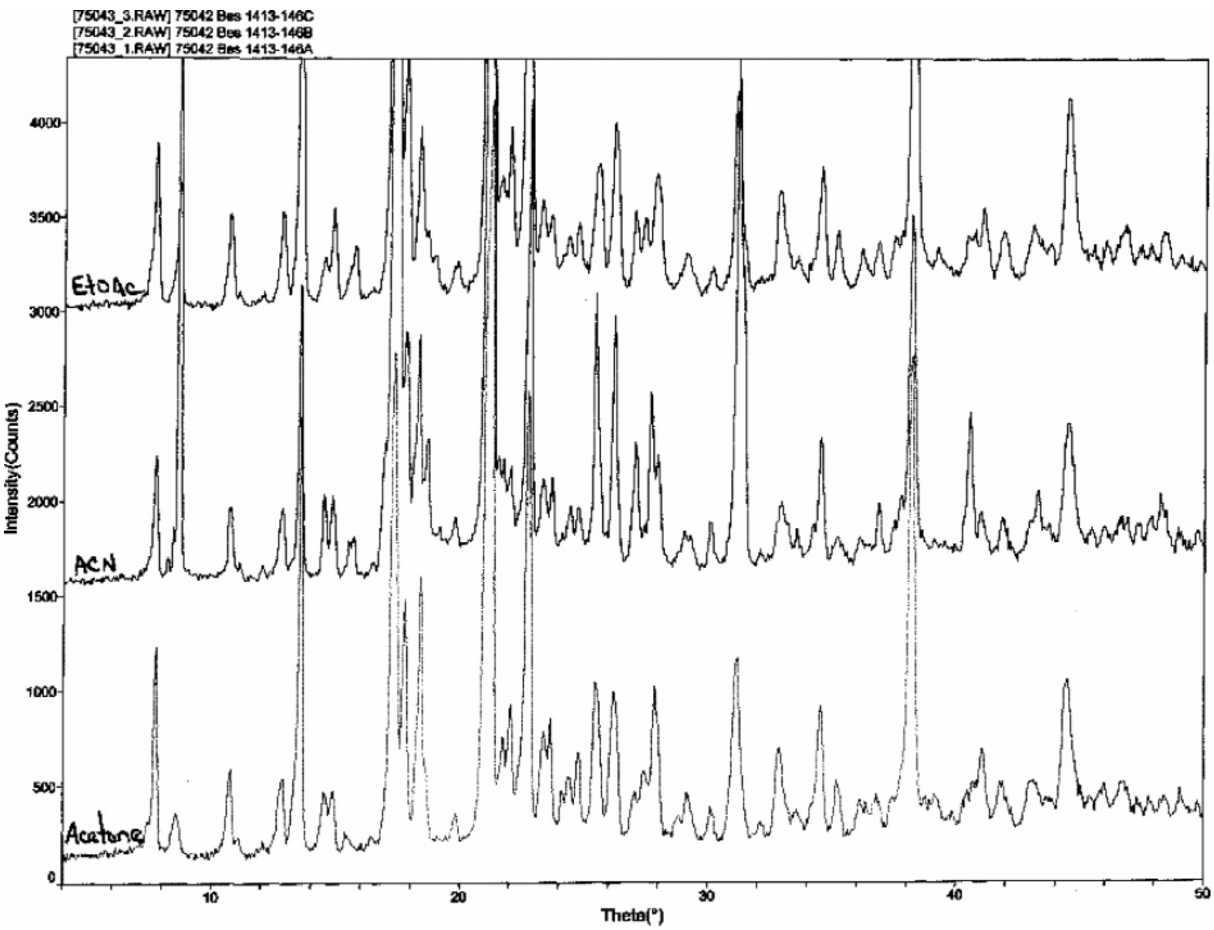

Figure 3. **PXRD of NBI-75042 besylate from several solvents.**

was verified at the time of analysis by spiking at the 2 ppm level. Details of the method are described in the Experimental Section.

Our concerns were legitimate as varying levels of benzene were detected in all of the commercial sources of BSA (Table 3) we procured. Testing a sample of ammonium benzenesulfonate did not find any benzene at detectable levels; however, project timing did not allow for a method to be developed for salt preparation with this as our BSA source. Timing and supplies of BSA also did not allow for us to set a raw material specification (i.e., below 2 ppm) for our upcoming campaign that would ensure no carryover to our product. Using a limit test for benzene as an in-process control, it was shown that none of the BSA salts that were prepared in our laboratories showed detectable levels of benzene (LOQ: 0.2 ppm) at or below our limit of quantitation (0.2 ppm) regardless of source.

For the GMP campaign, given the time constraints of the project, we were limited on sources of high purity BSA (>98%; tech grade was readily available in multikilogram quantity). Using BSA obtained from a different vendor for the small-scale work, a pilot/lab-scale run found the salt formed a thick gum. Investigation as to the cause of this revealed that the BSA sample contained ~12 w/w % water. Analysis of the BSA used for initial laboratory work found only 2.3 w/w % water by KF titration. When it was shown that the extra water was the cause of this effect, it was decided that an azeotropic removal of water, to <1%, would be incorporated into our manufacturing run with no additional demo batches. Details of the drying process are described in the Experimental Section. Much to our surprise, during release testing, a difference in the IR spectrum was observed for the GMP material when compared to our reference material. Analysis of the API revealed the forma-

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(12) There were small differences in the PXRD data presented in Figure 3: the peak at ~14.5 2θ is diminished in the ethyl acetate sample and the peak at ~41 2θ is increased in the acetonitrile sample. These changes are most likely due to particle size or orientation and do not suggest different polymorphs. Subtle shifts on the PXRD for different polymorphs may lead to processing challenges. See Remenar, J. F.; MacPhee, J. M.; Larson, B.; K.; Tyagi, V. A.; Ho, J. H.; McIlroy, D. A.; Hickey, M. B.; Shaw, P. B.; Almarsson, O. *Org. Process Res. Dev.* **2003**, *7*, 990. It is particularly useful to employ orthogonal methods of characterization when undertaking studies of polymorphism in pharmaceutical solids (see ref 9a). In addition to powder and single-crystal X-ray diffraction techniques, DSC, FTIR spectroscopy (see: Roy, S.; Atipamula, S.; Nangia, A. *Cryst. Growth Des.* **2005**, *5*, 2268−2276; Park, K.; Evans, J. M. B.; Myerson, A. S. *Cryst. Growth Des.* **2003**, *3*, 991−995), Raman spectroscopy (see: Wang, F.; Wachter, J. A.; Antosz, F. J.; Berglund, K. A. *Org. Process Res. Dev.* **2000**, *4*, 291−295), optical microscopy (see: Cashell, C.; Sutton, D.; Corcoran, D.; Hodnett, B. K. *Cryst. Growth Des.* **2003**, *3*, 869−872; Wahlstrom, E. E. *Optical Crystallography*, 5th ed.; J. Wiley and Sons: New York, 1979), and crystal modeling (see: Arslantas, A.; Ermler, W. C.; Yazici, R.; Kalyon, D. M. *Int. J. Mol. Sci.* **2005**, *6*, 291−302) are conventional techniques employed in characterizing polymorphism in pharmaceutical solids. Additional general texts for the characterization of pharmaceutical solids include (a) Byrn, S. R. *Solid-State Chemistry of Drugs*; Academic Press: New York, 1982. (b) Allen, L. V.; Popovich, N. G.; Ansel, H. C. *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, 8th ed.; Lippincott Williams and Wilkins: Baltimore, MD, 2005.

(13) *Guidance for Industry Q3C - Tables and Lists*; U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), ICH, revision 1: Washington, D.C., November, 2003.

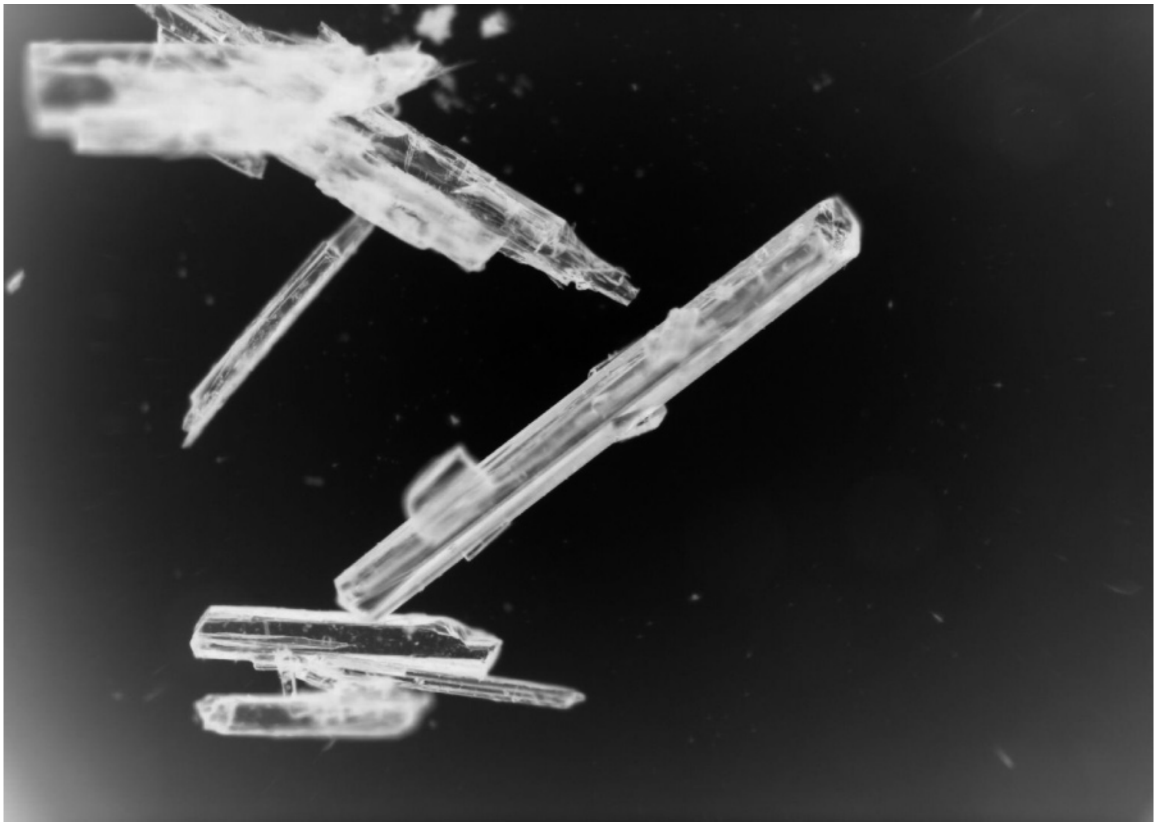

Figure 4. **Microscopic image (negative) of NBI-70542 besylate salt (from ACN).**

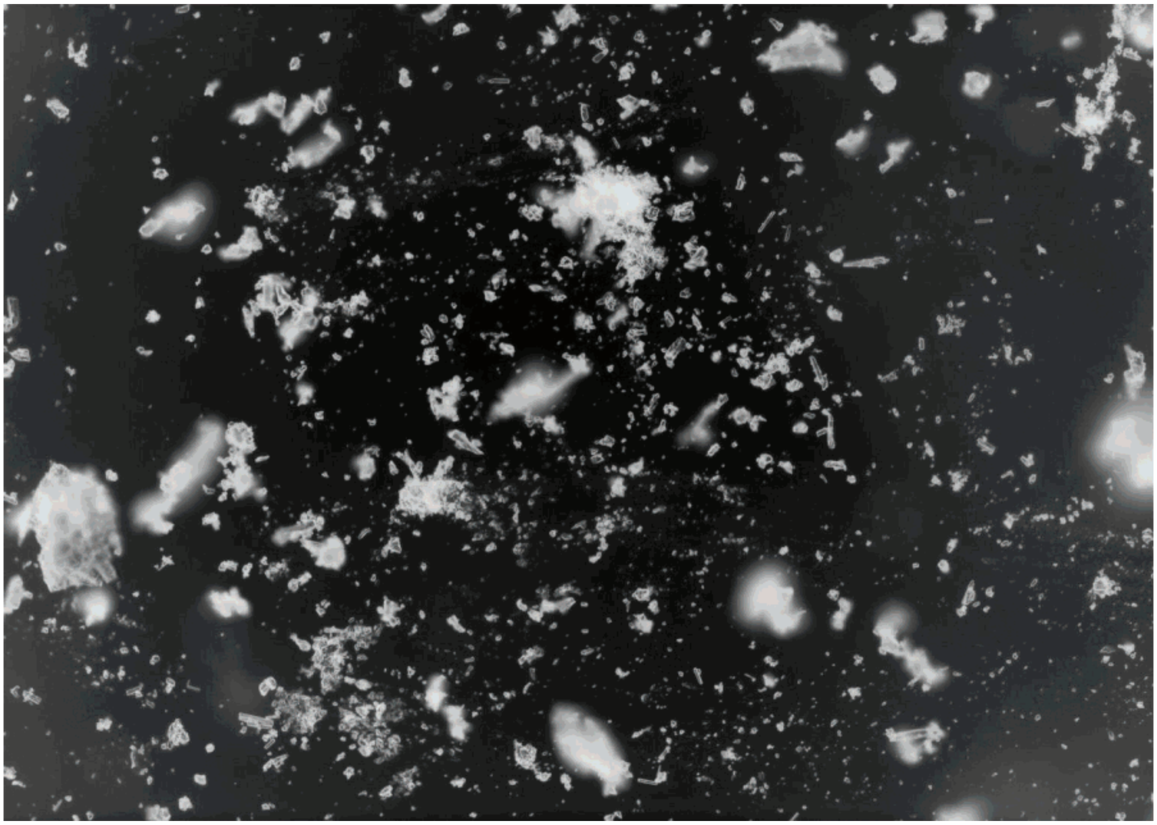

Figure 5. **Microscopic image (positive) of NBI-70542 tosylate salt.**

tion of a new polymorph. The polymorph formed in all of the previous work is referred to as "Form 1" and the newly formed polymorph as "Form 2". IR spectroscopy is able to distinguish between the two forms. Form 1 shows a strong stretch at 1244 cm^{-1} (Figure 7). This band is shifted to 1231 cm^{-1} in Form 2 (Figure 8). Additionally, the GMP material

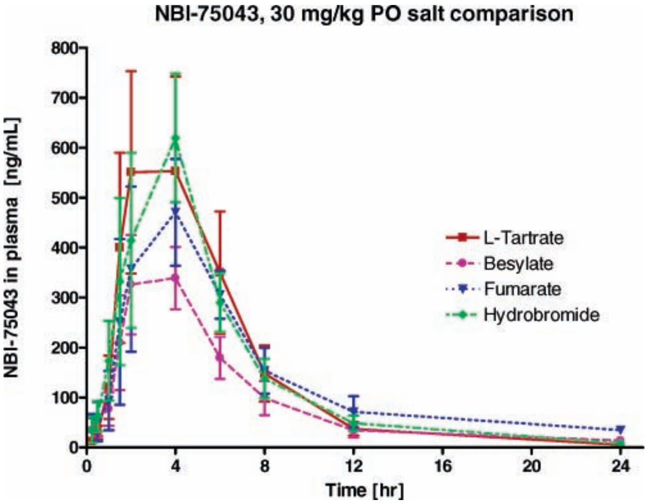

Figure 6. **Monkey PK salt comparison.**

Table 3. **Benzene content in benzenesulfonic acid**

source	lot	amount of benzene (ppm)
Acros	A0104995001	632
Aldrich	09130DA	703
Fluka	1216642	5858
Aldrich	09930HD	66
Kinbester	20060165	7

melted ~10 °C lower than the standard (Figure 9, 10) and under optical microscopy, the previously observed needles (Form 1, Figure 4) appeared primarily as plates (Form 2, Figure 11). PXRD (Figure 12) also confirmed the presence of a new polymorph, Form 2. To this point, all of the stability data on NBI-75043 besylate had been generated on Form 1. Rather than proceed with Form 2, we decided to consider converting it to Form 1 if possible, that is as soon as a method for its transformation could be determined.

Attempts at thermal conversion from Form 2 to Form 1 that were performed by dissolving the salt in hot solvents (ethyl acetate, isopropyl acetate, and chloroform[14]) followed by seeding and cooling resulted in a mixture of the two forms as observed by DSC and PXRD. The only change from our research-scale reactions to the manufacturing operations had been the necessary addition of the azeotropic drying steps. Several solvents (ethyl acetate, isopropyl acetate, THF, and MTBE) were spiked with water (0.5−2.0%), resulting in varying degrees of conversion. The most rapid conversion occurred in both tetrahydrofuran (THF) and ethyl acetate. We chose to focus on ethyl acetate; it was the solvent used for the initial crystallization, and therefore, it would introduce minimal changes to our process. Form 2 NBI-75043 BSA was slurried in ethyl acetate (10 volumes) with either 0.25% or 1.0% water at both room temperature and 50 °C overnight (17 h). The samples with 0.25% water showed incomplete conversion, whereas the samples with 1.0% water showed complete conversion to Form 1. Additional experiments demonstrated that complete conversion to Form 1 occurred within 30 min at 50 °C. We knew from the pilot run that too much water would cause gumming and chose not to experiment with greater amounts of water. Using these conditions, we were able to convert Form 2 to Form 1, as confirmed by IR and PXRD. DSC analysis, on the other hand, was less clear. When converting Form 2 to Form 1, there is a clear shift of ~10 °C higher in the observed endotherm; however, there is considerable lot-to-lot variation in endotherm (~15 °C). Additionally, our reference lot had a very sharp onset to its melt, while the onset for Form 2 was broader. In samples of Form 1 that were converted from Form 2, the onset was broader than our standard (Figure 9 vs Figure 13). This was observed irrespective of whether the lots were converted via slurry or dissolved and recrystallized. It should also be noted that this was observed despite the fact that no evidence of Form 2 existed by IR or PXRD analysis.

With a method of polymorph conversion in hand, we next sought to understand why Form 2 was produced. One of the reasons we selected the BSA salt was that it only produced one polymorph from several solvents (acetonitrile, acetone, ethyl acetate; see Figure 3). While the formation of Form 2 was unexpected, we suspected that water content was the key to its formation. This theory is what led to our method of conversion from Form 2 to Form 1. In order to demonstrate the role of water in polymorph formation and control, a portion of benzenesulfonic acid was dried azeotropically with ethyl acetate six times until it measured 0.45% water by KF analysis. This material was used for a small-scale salt formation (1.6 mmol). Analysis by DSC (Figure 14) and PXRD (Figure 15) showed that Form 2 was formed predominantly, thereby verifying our hypothesis.

With an explanation for the formation of Form 2, we could avoid it in future lots, and with a method for its conversion to Form 1 in hand, our focus turned back to our manufacturing campaign. One kilogram of the GMP batch was dispositioned as “not for clinical use” and reworked as detailed in the Experimental Section in order to gain experience with this conversion at a meaningful scale. The conversion was complete in <2 h with excellent recovery (96%) and excellent purity (99.8%). Following this procedure, the rest of the GMP batch was converted from Form 2 to Form 1 efficiently.

Conclusions

We have described the difficulties encountered with the first salt form of NBI-75043. Accordingly, a decision tree to rapidly and efficiently find a new salt form was developed and applied to this specific example. We were able to overcome unexpected polymorph formation encountered during the first manufacturing campaign for the new salt.[15] Future development work should include determining the operational limits of both solvents and acid in terms of purity

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(14) The feasibility of thermal conversion was under investigation. Chloroform, a class 2 solvent was selected as it possesses vastly different properties than the esters. Had thermal conversion been successful, a search for alternate solvents would have been performed.

(15) A similar conversion of an API salt to a more stable form by controlling water to >0.2% has been reported by Grosso, J. A. U.S. Patent 5,162,543, 1992. Ethanol/water, 95:5, is the solvent most often used for salt formation when performing optical resolutions (Jaques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolutions*; Wiley: New York, 1981; pp 381−395). Its inclusion in a solvent screen may be helpful in identifying hydrated forms.

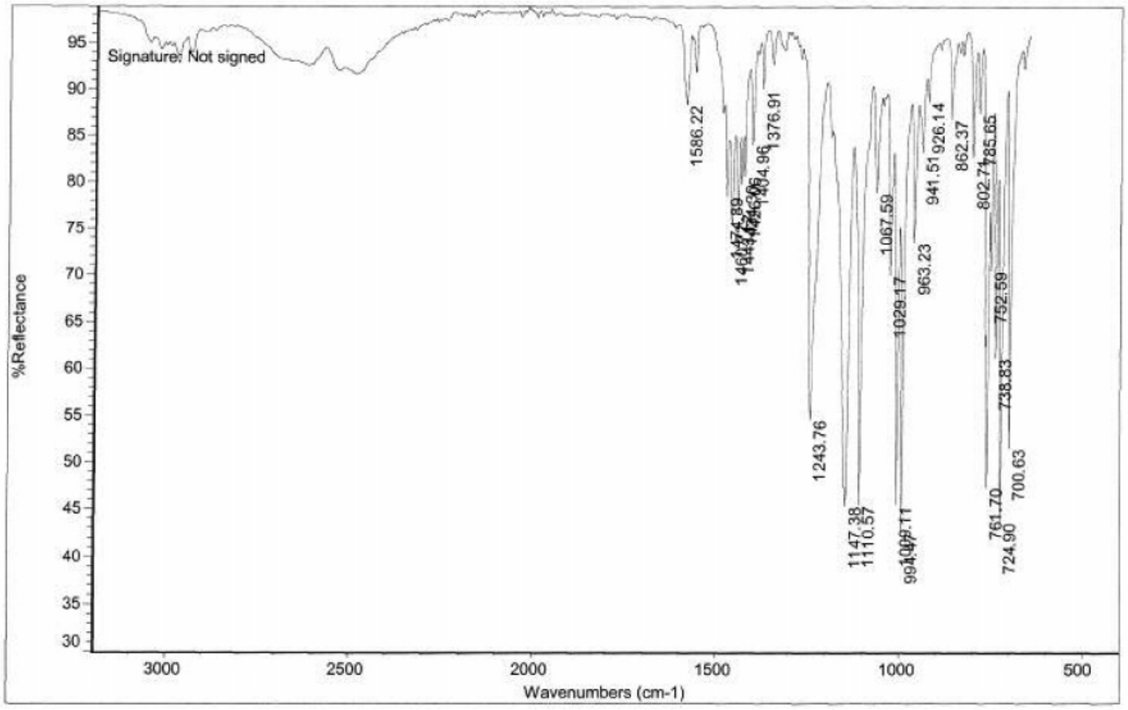

Figure 7. **IR spectrum of NBI-75043 besylate Form 1.**

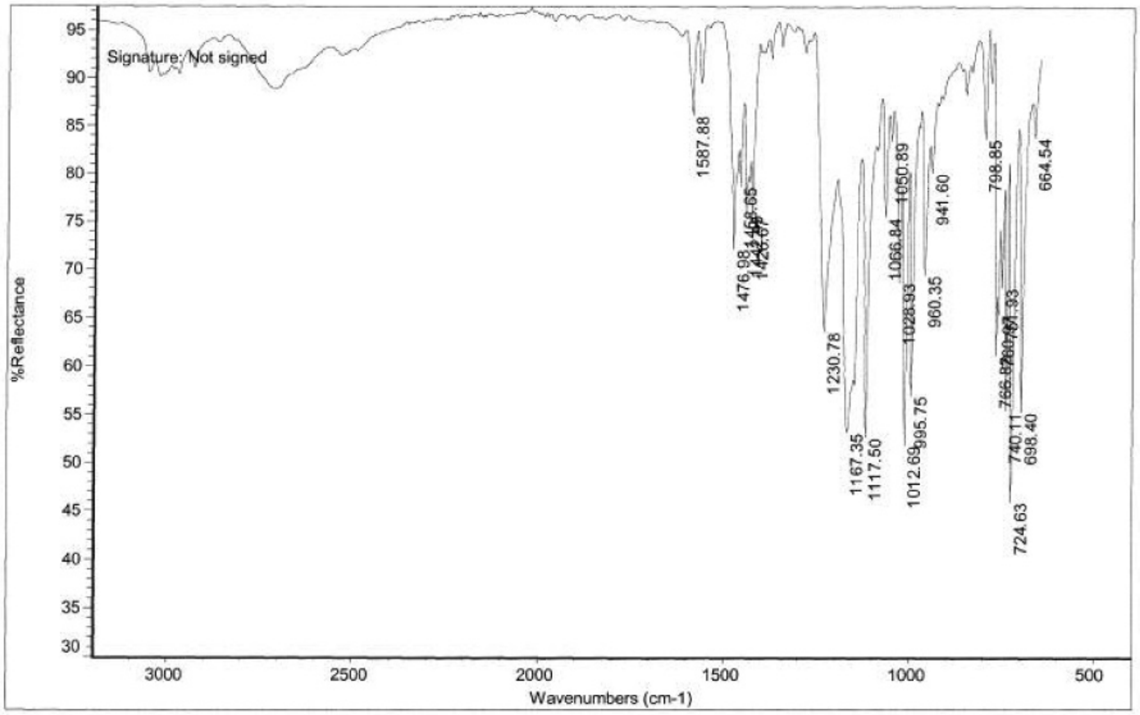

Figure 8. **IR spectrum of NBI-75043 besylate Form 2.**

and water content. Additionally, we have been reminded again that the number of polymorphs for any given compound is $N + 1$, where $N =$ the number of known polymorphs; hence, vigilance is always necessary.[16] As shown in this case study, having an option to rework the final product through a simple but robust procedure is always a great advantage. Again, it is shown that a thorough salt screening/selection and polymorph study should be performed as early as possible in the drug development process. Ideally, this should be conducted no later than declaration of a development drug candidate.

Experimental Section

DSC measurements were performed on a Mettler Toledo DSC822^{e} equipped with a Mettler Toledo TS0801RO sample robot using 40-μL aluminum pans, from 25 to 300 °C at 10

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(16) McCrone, W. C. *Physics and Chemistry of the Organic Solid State*; Wiley-Interscience Publishers: New York, 1965; pp 725−767.

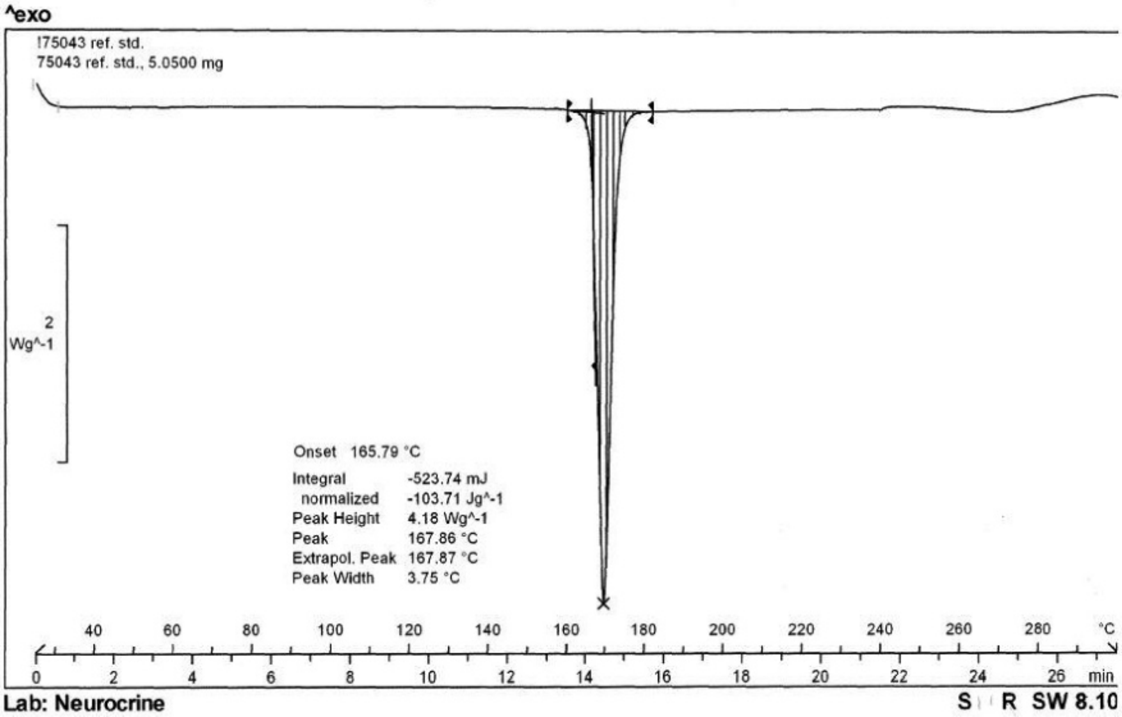

Figure 9. **DSC thermogram of NBI-75043 besylate Form 1.**

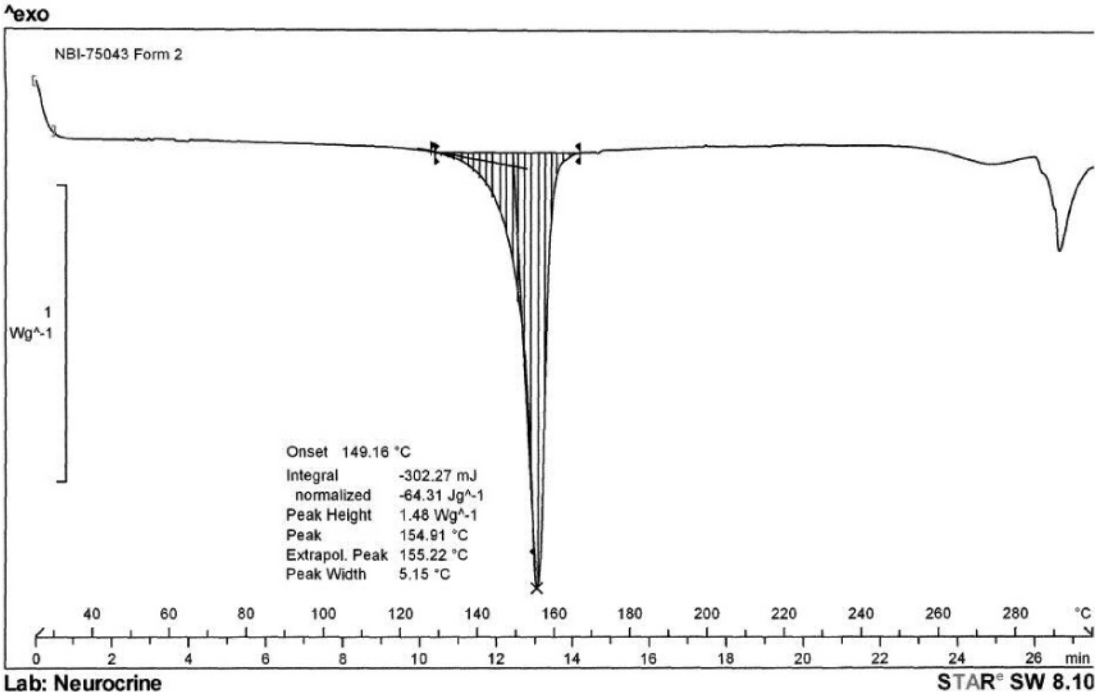

Figure 10. **DSC thermogram for NBI-75043 besylate Form 2.**

°C/min. TGA analysis was determined with a TA Instruments Hi-Res TGA 2950 thermogravimetric analyzer. KF titration was performed with a Brinkmann 756 KF coulometer with a 703 Ti stand or a Mettler Toledo DL31 Karl Fischer titrator. DVS analysis was performed on an SMS Surface Measurement Systems DVS Dynamic Vapour Sorption DVS-1. PXRD spectra were obtained with either a PANalytical X'pert pro or Rigaku miniflex spectrometer. IR spectra were obtained with a Thermo Nicolet Nexus 470 FT-IR E. S. P. Optical observations were acquired with a Leica DMLP light microscope with hot stage and CCD.

Methods

HPLC purity/assay: mobile phase A = 0.1% formic acid in water, pH adjusted to 2.0 with HCl.

Mobile phase B = 90% methanol, 10% acetonitrile.

Sample diluent = 95% A, 5% B.

Column = Phenomenex Luna C8(2), 4.6 mm × 150 mm, 3 μm particle size.

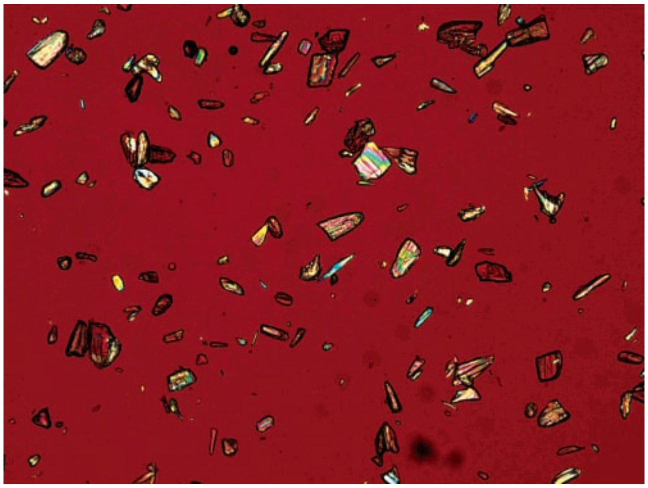

Figure 11. **Microscopic image of NBI-75043 besyalte Form 2.**

Injection volume = 15 μL.
Detector = DAD; λ = 260 nm.
Flow rate = 1.0 mL/min.
Column temperature = 50 °C.

linear gradient:	time	%A	%B
	0.0	95	5
	5.0	85	15
	15	75	25
	29.5	10	90
	31.0	5	95
	34.5	5	95
	35	95	5
	40	95	5

Sample prep at 0.2 mg/mL NBI-75043 in diluent.

GC benzene: Agilent 6890 GC with Agilent 5973 MSD, helium carrier gas.

Column: DB-624, 60 m × 320 μm, 1.8 μm film thickness.

Oven: 35 °C (5 min hold), up to 210 °C @ 20 °C/min (3.25 min hold), total run time = 17 min.

Injector: 180 °C, split ratio 10:1, constant helium flow through column at 1.5 mL/min.

Detector: MSD, single ion monitoring (SIM) for mass 78, EM voltage =1700, source temp 230 °C, quadropole temp 150 °C.

Sample prep: the sample was dissolved in CH_2Cl_2 and 1μL injected. If the sample was aqueous, the sample was extracted using CH_2Cl_2 by vortexing. An internal standard, ethyl benzene, was used when performing the extractions.

Salt Screen. *Citrate Salt Procedure.* To a 25-mL round-bottom flask was charged NBI-75042 free base (1.0 g, 3.2 mmol, 1.0 equiv) and ethanol (6.6 mL), and the flask was swirled to dissolve NBI-75042. Citric acid (619 mg, 3.2 mmol, 1.0 equiv) was added, and the flask was swirled to dissolve. The mixture was briefly heated, with a heat gun if necessary, to affect complete dissolution. The flask was held at room temperature for several hours with no crystallization observed. The flasks were concentrated in vacuo to a solid.

Solubility Screen. To each of 10 1-dram vials was charged ~50 mg of NBI-75042 citrate salt. The salts were just covered with solvent (3−5 drops). The solvents used were water, methanol, ethanol, isopropanol, acetone, THF, ethyl acetate, acetonitrile, methyl *tert*-butyl ether, and hexanes. The vials were tightly capped and heated with a heat gun to dissolve the solids. The vials were allowed to

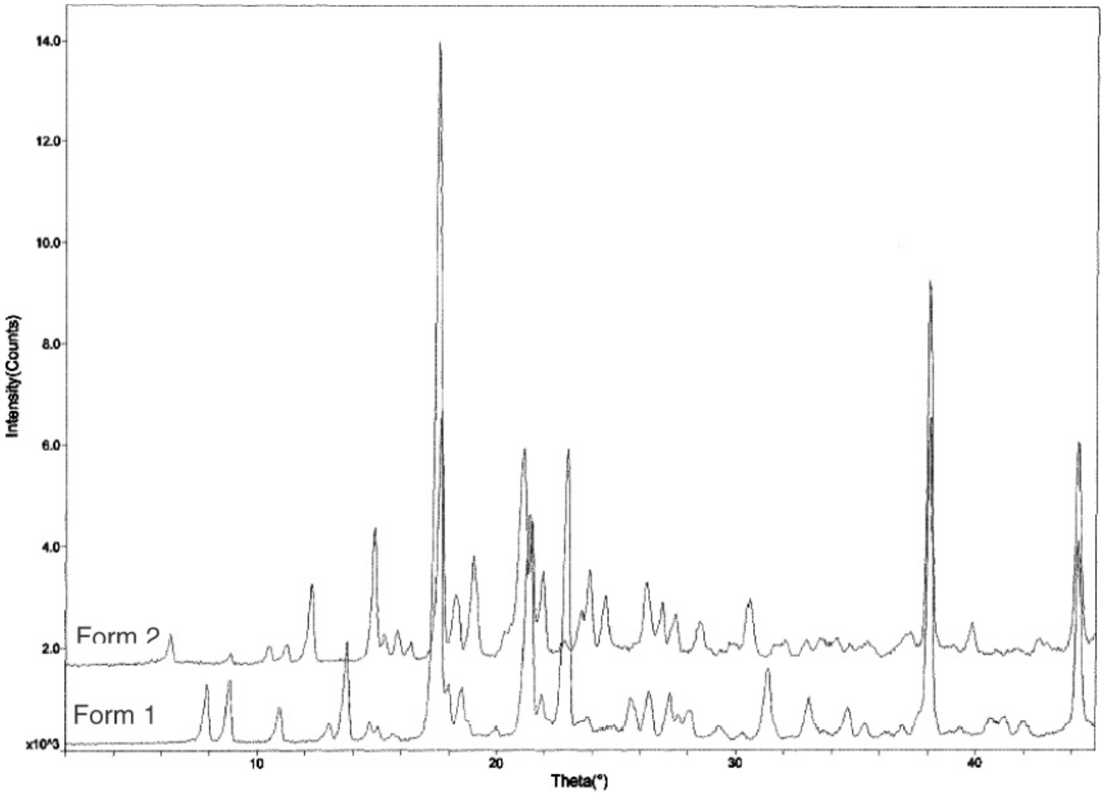

Figure 12. **Comparative PXRD patterns for NBI-75043 besylate Form 1 and Form 2.**

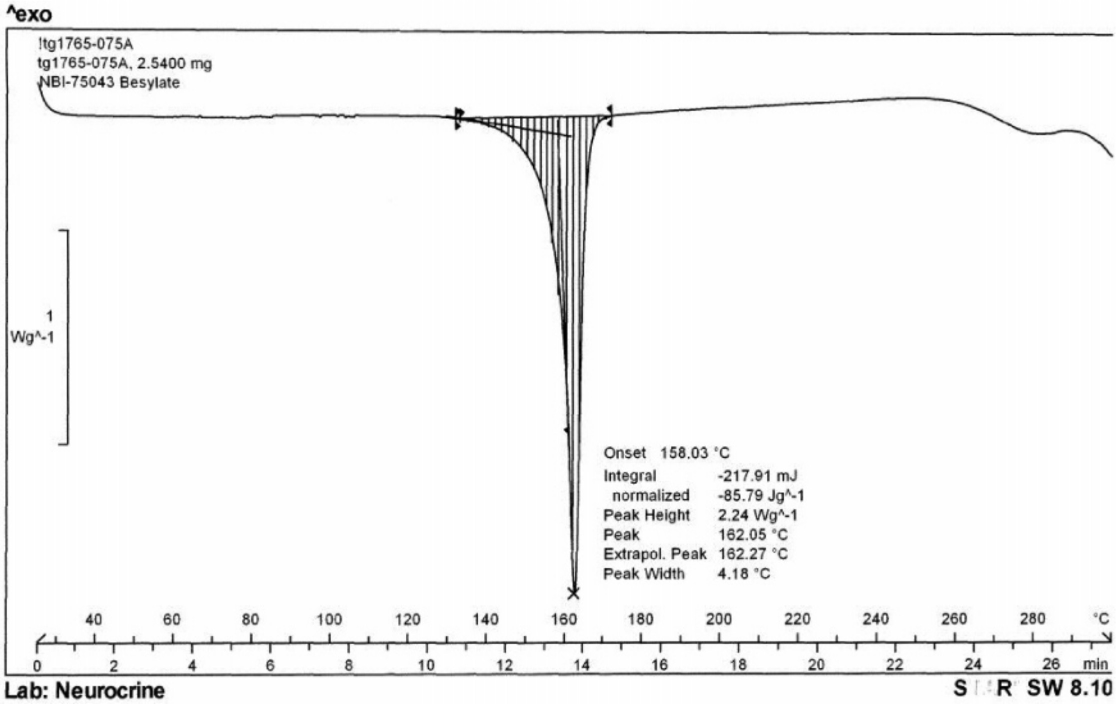

Figure 13. **DSC thermogram of NBI-75043 besylate Form 1 converted from Form 2.**

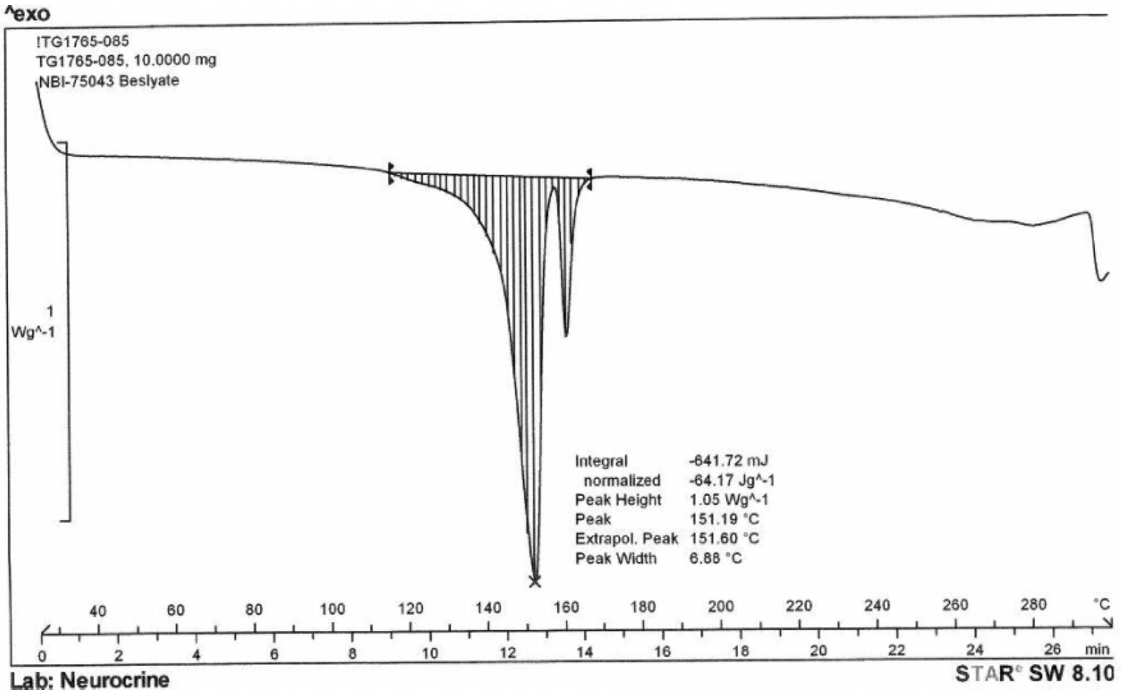

Figure 14. **DSC thermogram of NBI-75043 besylate Form 2 produced from dry BSA.**

cool to room temperature and to stand for a period of ∼18 h. Material that appeared to have crystallized was filtered, air-dried, and analyzed by DSC.

Small-Scale Salt Formation. A 1-L Morton flask equipped with a mechanical stirrer and thermocouple was charged with NBI-75043 (10.02 g, 32 mmol, 1.0 equiv) and ethyl acetate (300 mL). The reactor was stirred and heated to 65 °C (internal temperature). A solution of BSA (5.05 g, 32 mmol, 1.0 equiv) dissolved in ethyl acetate (23 mL) was added over 1 min. The solution became cloudy as the last ∼4 mL of solution was added. Heating was discontinued, and the reactor was allowed to slowly cool to room temperature (22 °C) overnight (∼16 h). The material was filtered on a Büchner funnel (Whatmann no. 4 paper), and a portion (∼100 mL) of the mother liquor was used to rinse the reactor and the filter cake. The cake was air-dried for ∼20 min, and then it was transferred to a vacuum oven and dried overnight (50 °C, 8 Torr, 18 h) to produce 14.66 g, 31 mmol, 97% yield of a white powder.

Large-Scale Salt Formation. BSA (3.6 kg, 22.8 mol, 1.1 equiv) was charged to a 20-L rotavap flask. Ethyl acetate (7 kg) was added, the flask was attached to the rotavap, and

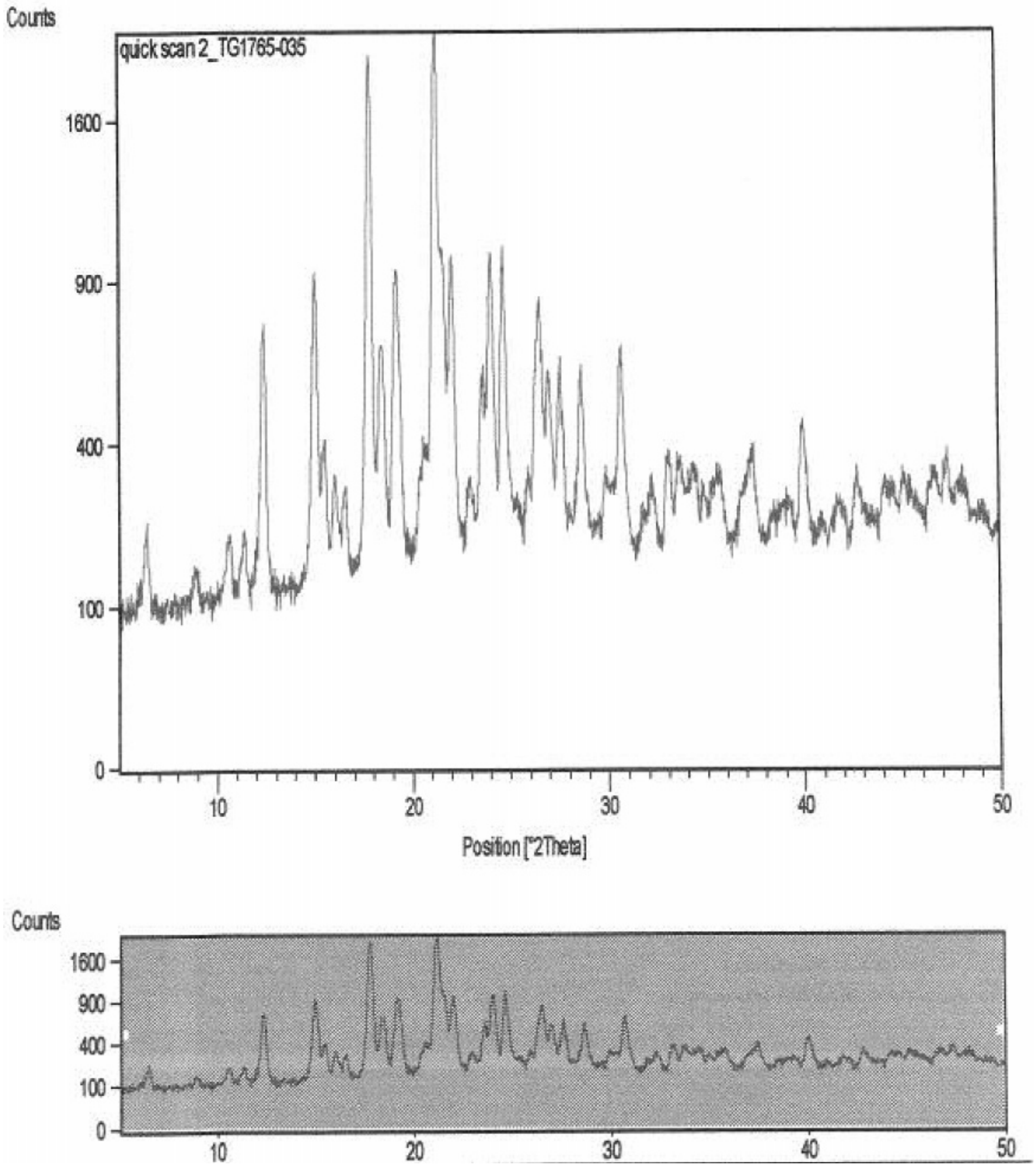

Figure 15. **PXRD diffraction pattern from NBI-75043 besylate Form 2 produced from dry BSA.**

the mixture was concentrated to dryness without the bath temperature exceeding 50 °C. This process was repeated an additional two times with ethyl acetate (7 kg) each time. The contents of the flask were dissolved in ethyl acetate (14 kg), and a portion was analyzed by KF. Water was found at 2.28%, and the flask was concentrated to dryness again, followed by addition of ethyl acetate (7 kg), concentration to dryness, and dissolving the contents in ethyl acetate (14 kg). After KF analysis, water was found at 0.63%, and the material was deemed dry enough to continue (<1.0%).

A 400-L glass-lined reactor was charged with a solution of NBI-75043 (6.4 kg, 20.6 mol, 1 equiv) in ethyl acetate (7.5 kg). The reactor was charged with additional ethyl acetate (126.5 kg). The reactor was heated to 65 °C. The solution of BSA was charged to the reactor; the flask was rinsed with additional ethyl acetate (3 kg) which was also charged to the reactor. The reactor was cooled to 20 °C over 4 h, and the contents were filtered through a Nutsche filter. The reactor was rinsed with additional ethyl acetate (10 kg) and the filter cake blown dry with nitrogen for 20 min. The cake was transferred to vacuum trays, transferred to a vacuum oven, and dried to constant weight at a temperature ≤55 °C to yield 8.15 kg, 84.0%.

Conversion of Form 2 to Form 1. A 22-L Morton flask equipped with a mechanical stirrer and thermocouple was charged with NBI-75043 BSA Form 2 (1 kg) and ethyl acetate (10 L). The reactor was stirred, and heating to 50 °C (internal temperature) was initiated. While the reactor was heating, water (200 mL, 2.0% in EtOAc) was added. An immediate change in the consistency of the suspension was observed. The fine suspension took on a granular appearance; at the same time, an endotherm was observed with the temperature of the reaction dropping from ∼23 °C to ∼18 °C. The reaction was heated to 50 °C (internal temperature) over ∼2 h. A sample (∼50 mg) was withdrawn, filtered, air-dried for ∼20 min, and analyzed by DSC which indicated disappearance of Form 2. Stirring was continued, and the reactor was allowed to cool to room temperature overnight. The room-temperature suspension (22 °C) was filtered, and the solids were air-dried for 30 min and then dried in a vacuum oven (∼8 Torr, 50 °C, 16 h) until constant weight was achieved; 964.4 g, 96% recovery.

Acknowledgment

We thank Richard Moore, Brigit Durell, and Ajay Madan for helpful discussions. We also thank Daniel Bonneville and Elina Simberg for PXRD and IR data.

Received for review October 25, 2006.

OP060221A