Scientific paper

First Direct Isolation of Stable α-Form Crystals of Mirabegron, a Selective β_3-Adrenoceptor Agonist

Dattatray G. Deshmukh,[1,2] Mukund N. Bangal,[1] Anil C. Mali,[1] Vijay J. Medhane[2] and Vijayavitthal T. Mathad[1,*]

[1] *Department of Process Research and Development, Megafine Pharma (P) Ltd., 201, Lakhmapur, Dindori, Nashik-422 202, Maharashtra, India.*

[1] *Organic Chemistry Research Center, Department of Chemistry, K. T. H. M College, Nashik-422 002, Maharashtra, India.*

** Corresponding author: E-mail: vt.mathad@megafine.in, drvtmathad@yahoo.co.in*

#Megafine Publication Number: MF/026/2016

Received: 17-02-2017

Abstract

An efficient and scalable method for the direct isolation of stable α-form crystals of Mirabegron (**1**) is developed. The developed method negates transformation of metastable β-form crystals into α-form crystals thereby overcoming the limitations of reported methods and avoids additional processing steps during its manufacture. The developed method directly provides stable α-form crystals of Mirabegron (**1**) with yield of around 84% and purity of >99.77% by HPLC in a single step.

Keywords: β_3-adrenoceptor agonist, Mirabegron, polymorphism, polymorphic transformation.

1. Introduction

Crystallization operation is often critical in pharmaceutical industries as it solely determines product properties such as the polymorphism, crystal size distribution, and crystal habit.[1] Change in polymorphic form may alter product characteristics such as dissolution, hardness, color, optical properties, melting point or chemical reactivity. As regulatory perspective, developing the process which provides exclusively pure polymorph of a drug substance that should be stable enough to maintain the polymorph integrity during formulation of the drug product and storage throughout its life cycle is very necessary. The driving force in crystal formation is super saturation and when a super saturation of crystallizing compound is created by chemical reaction, the operation is known as reactive crystallization. In reactive crystallization, reactions can be very fast compared to the mass transfer rates and growth rates to the crystals thus reactive crystallizations can lead to the exclusive formation of the metastable polymorph of a system.[2] This article is aimed to provide a case study wherein systematic crystallization of stable α-form crystals of Mirabegron (**1**) is achieved by circumventing the following limitations of the known processes; (a) reactive crystallization of metastable β-form crystals of **1** and (b) transformation of metastable β-form crystals into α-form crystals using a seed.

2. Background

Mirabegron (**1**), chemically known as 2-(2-amino-1,3-thiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide is a selective agonist for the human beta 3-adrenoceptor[3] (β_3-AR) approved for the symptomatic treatment of urinary urgency, increased micturition frequency and/or urgency incontinence in patients with overactive bladder (OAB) syndrome.[4–5] Mirabegron has distinct and novel mechanism of action compared to antimuscarinics[6] as it improves the storage capacity of the bladder without inhibiting bladder voiding thereby prolonging the time between trips to the toilet for the patient.[5] Mirabegron (**1**), developed by Astellas Pharma was approved by the USFDA in 2012 and EMA in 2013

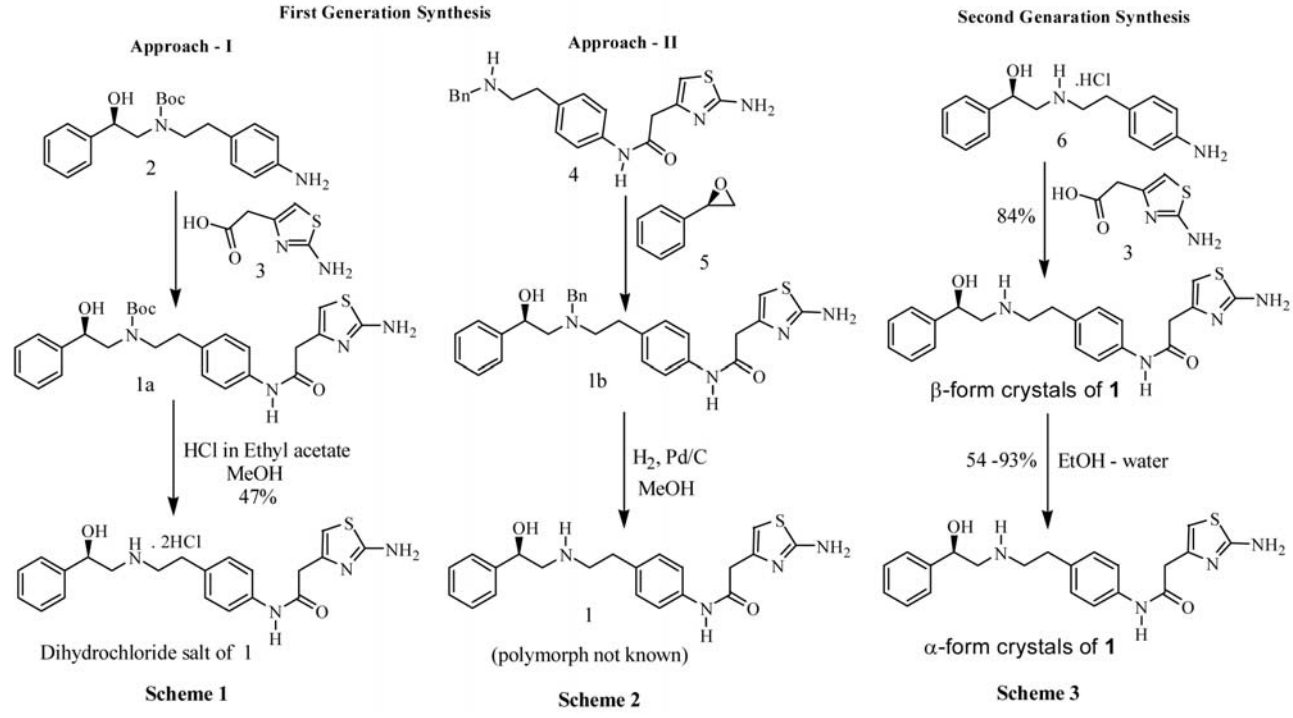
and is currently available in the market under the trade name Myrbetriq® in the US and Betmiga® in Europe.[8]

According to the literature reports Mirabegron free base exhibits polymorphism and is know to possess two crystalline forms, namely α-form and β-form crystals. The β-form crystals of mirabegron is hygroscopic and tends to gain water content up to 3% under the relative humidity of about 20%, whereas α-form crystals did not show any increase in the water content over the entire range of relative humidity from 5% to 95% concluding that α-crystal form is stable and suitable for use as a medicine.[9] According to EMA assessment report, Betmiga formulation is known to contain α-crystalline form of mirabegron free base.[5]

The first generation syntheses[10] discloses two synthetic approaches for **1** (Schemes 1 and 2). The first approach (Scheme 1) involves condensation of Boc-protected aniline **2** with thiazole acid **3** to obtain Boc-protected intermediate **1a**. The Boc group was de-protected in **1a** using HCl to furnish di-hydrochloride salt of **1** with an overall yield of about 26% over two stages. However, this report does not exemplify the isolation of free base of mirabegron (**1**).

The second approach (Scheme 2) mentioned in the report is shuffling of synthetic steps of Scheme 1. In the second approach, benzyl (Bn) protected secondary amine **4** was reacted with (*R*)-styrene oxide (**5**) to provide Bn-protected intermediate **1b**. However, detailed synthetic procedure is not provided in the report for this approach. Neither of these approaches have provided procedure for preparing mirabegron free base (**1**) which has been used to formulate drug product and clarity on polymorphic forms associated with free base.

Second generation synthesis[9] (Scheme 3) reported for **1** involves coupling of **6** with **3** followed by addition of aqueous sodium hydroxide solution to provide β-crystal form directly from the reaction mass through reactive crystallization. β-Form crystals was then re-crystallized in subsequent stage in aqueous ethanol using seeds of α-crystals to provide α-crystal form with 74–78% yield over two stages. Though second generation synthesis is improved over first generation but has a limitation to perform additional manufacturing step of converting β-form crystals into α-form crystals using seeds of α-crystals. This additional step to achieve α-form decreases the efficiency in terms of yield loss and productivity. However reported transformation of β-form crystals to α-form crystals lack consistency in providing pure α-form crystals at our end. Several other known methods for the preparation of α-form involves additional crystallization of the isolated material using various solvent systems.[11] Most importantly the desired crystals achieved via polymorphic transformation generally pose increased risk of polymorph contamination (polymorphic purity) during the manufacturing process as well as during its stability studies. Thus, it may be difficult to maintain the integrity of the polymorphs which are achieved via transformation of metastable form / pseudo form. This triggered us to aim for the process which could directly produce α-form crystals selectively and directly through a proper solvent-mediated crystallization process. The details of process optimization work carried out in providing a single stage met-



Schemes 1–3. Reported syntheses of mirabegron (**1**).

hod for direct isolation of α-form crystals which is suitable for industrial scale are discussed herein.

3. Results and Discussion

Our initial approach to achieve α-form crystals obviating the polymorphic transformations was to replace the reported reaction medium (water) with a suitable organic solvent. The purpose of replacing the water with organic solvent before neutralizing the reaction mass was to avoid the reactive crystallization and to retain the product in solution phase. Among the several solvents explored for the reaction, methanol showed moderately good conversion of **6** to **1** in solution state. The reaction mixture was then neutralized with aqueous solution of sodium hydroxide as base and obtained clear solution was concentrated to provide crude residue, which was dissolved in organic solvent, filtered to remove the insoluble inorganic material and crystallization of **1** was explored using various solvents and anti-solvents, but the approach was unsuccessful as it provided impure and hygroscopic solids.

In our next approach, water was retained as a reaction solvent and water immiscible organic solvent was added to the reaction mass prior to basification to extract the product into the organic phase to arrest reactive crystallization of β-form. The organic phase containing the product was then separated and crystallization was attempted using anti-solvent(s) preferentially to achieve pure α-form crystals of **1**. Among several solvents explored for extraction, ethyl acetate as a solvent and *n*-heptane, toluene, MIBK, DIPE as anti-solvents exclusively provided pure α-crystalline form. However, this combination of solvent and anti-solvents failed to provide chemical purity as per ICH[12] requirement. Additionally, poor solubility of **1** in ethyl acetate necessitated huge volume of ethyl acetate (around 25 volumes/g of **6**) for complete extraction of **1** even at the elevated temperature. The yields obtained with this approach were also not satisfactory. Attempts to achieve yield and ICH quality material by judicious choice of anti-solvents were not successful. The results of the various combinations of solvent(s) and anti-solvent explored in this approach are provided in Table 1.

Table 1. Screening of solvents and anti-solvents for isolation of α-form crystals of **1**.

Entry	Solvent[1] (vol[#])	Co-solvent[2] (vol[#])	Anti-solvent (vol[#])	Anti-solvent addition temp(°C)	Yield (%)	Purity by HPLC (area %) 1	6	3	SMI*	Crystal form
1	2-MeTHF (30)	–	*n*-heptane (80)	25–30	21.6	99.37	ND	0.01	0.13	α+β
2	DCM (20)	–	*n*-heptane (10)	25–30	59.7	97.63	0.05	0.04	1.02	α
3	EA (25)	–	*n*-heptane (20)	55–60	78.3	97.42	0.16	ND	1.02	α
4	EA (25)	–	PhMe (20)	60–65	60.1	99.61	0.06	ND	0.07	α
5	EA (25)	EtOH (5)	*n*-heptane (15)	70–75	77.4	99.32	0.17	ND	0.17	α
6	EA (25)	IPA (10)	*n*-heptane (25)	55–60	71.1	99.40	0.07	ND	0.16	α
7	EA (25)	MeCN (5)	*n*-heptane (20)	55–60	53.5	99.65	0.06	ND	0.04	α
8	EA (25)	Me_2CO (10)	*n*-heptane (30)	45–50	70.9	99.75	0.04	ND	0.10	α
9	EA (25)	Me_2CO (5)	PhMe (30)	45–50	68.3	99.56	ND	ND	0.17	α
10	EA (25)	Me_2CO (5)	DIPE (30)	45–50	70.2	99.58	ND	ND	0.18	α
11	EA (25)	Me_2CO (5)	MIBK (30)	45–50	46.4	99.69	ND	ND	0.11	α

2-MeTHF = 2-methyltetrahydrofuran, DCM = dichloromethane, EA = ethyl acetate, EtOH = ethanol, IPA = isopropyl alcohol, MeCN = acetonitrile, Me_2CO = acetone, PhMe = toluene, DIPE = diisopropyl ether, MIBK = methyl isobutyl ketone; [#]volumes of solvent and anti-solvent are with respect to quantity of **6**. solvent[1]: solvent used for extraction; co-solvent[2]: solvent added after extraction of product to enhance the HPLC purity of **1**; *SMI: single maximum impurity; isolation of **1** in entry 1 to 11 is carried out by filtration at 25–30 °C.

Table 2. Optimization results of anti-solvents with *n*-butanol with respect to their volumes, impurity profile and yields of α-form crystals.

Entry	Solvent (vol[#])	Anti-solvent (vol[#])	Anti-solvent addition temp (°C)	Purity by HPLC (area %) 1	6	7	8	SMUI	9 (ppm)	10 (%)	Yield (%)	Crystal form
1	*n*-butanol (10)	*n*-heptane (20)	75–80	96.87	0.13	0.27	ND	1.53	NA	NA	69.7	α
2	*n*-butanol (12)	toluene (12)	65–70	99.79	0.02	0.05	ND	0.06	NA	0.01	71.9	α
3	*n*-butanol (6)	toluene (14)	65–70	99.77	0.02	0.05	ND	0.07	NA	0.01	84.9	α
4	*n*-butanol (6)	toluene (14)	65–70	99.78	0.01	0.07	ND	0.07	NA	0.04	84.6	α
5	*n*-butanol (6)	toluene (14)	65–70	99.77	0.01	0.10	ND	0.04	ND	ND	81.1	α
6	*n*-butanol (6)	toluene (14)	65–70	99.86	0.02	0.03	ND	0.02	16	ND	83.9	α

Note: Entries 3 to 6 are carried out under the same process conditions; ND: Not detected; NA: Not analyzed; [#]volumes of solvent and anti-solvent are with respect to quantity of **6**; Isolation of α-form crystals of **1** in entry 1 to 6 is carried out by filtration at 25–30 °C; SMUI: single maximum unknown impurity.

To overcome the issues associated with ethyl acetate, extraction of **1** was attempted with an amphiphilic solvent that was polar enough to solubilize the molecule but had enough hydrophobicity to have very limited miscibility with water. *n*-Butanol has all the attributes that we were looking in a solvent to be used for extraction of **1**. The exploratory reactions using *n*-butanol (Table 2; entries 1 and 2) furnished **1** with comparable yields and purity to those obtained with ethyl acetate. More importantly, only 10–12 volumes of *n*-butanol were sufficient to extract as opposed to 25 volumes of ethyl acetate. In addition to this, the extraction of **1** could be carried out at room temperature. This made *n*-butanol the perfect choice since it hit the 'trifecta' for all that was desired in the solvent of choice. Among the two preferred anti solvents (*n*-heptane and toluene), toluene furnished extremely promising results with *n*-butanol with respect to chemical purity and yield as shown in Table 2.

Further, to improve the yield of **1**, around half of the volume of *n*-butanol used for the extraction was concentrated *in vacuuo* below 70 °C before the toluene was added as anti-solvent. The mixture was gradually cooled to 25–30 °C, precipitated solid was filtered and dried under vacuum at 55–60 °C to get **1** with 84% yield and 99.86% purity by HPLC. Around 10% yield was increased without compromising on stringent quality requirements with this modification. The trend data of yield, purity and polymorphic form for kilogram-scale batches is provided in Table 2 (entries 5 and 6).

The schematic representation of single step process for achieving the α-form crystals is provided in Scheme 4 and structure of impurities controlled during the crystallization process are provided in Figure 1.

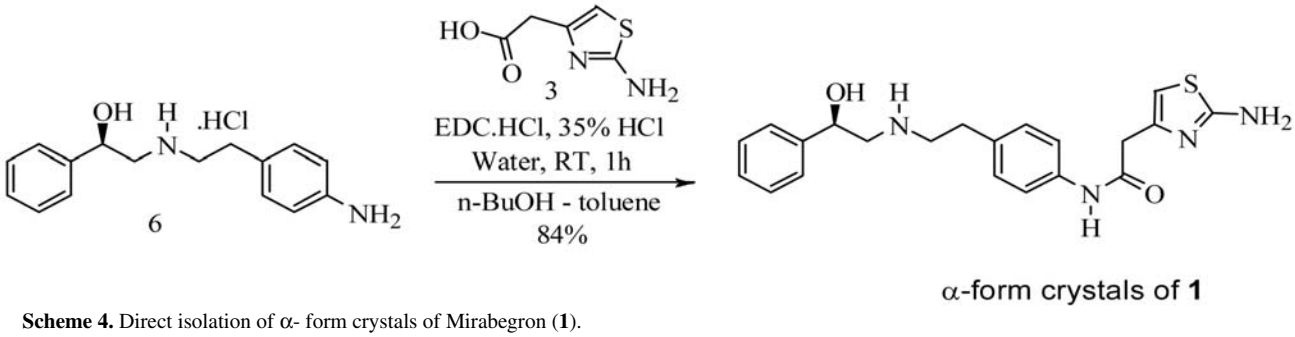
4. Conclusion

In conclusion, an efficient and robust method for direct isolation of the α-form crystals of mirabegron (**1**) has been developed using *n*-butanol mediated work-up. The developed process provided α-form crystals obviating the subsequent recrystallization step necessitated by earlier methods with an overall yield of around 84% and purity of around 99.77% by HPLC.

5. Experimental Section

5. 1 General

All reagents, solvents, and processing aids are commercial products and were used as received. For reactions



Scheme 4. Direct isolation of α- form crystals of Mirabegron (**1**).

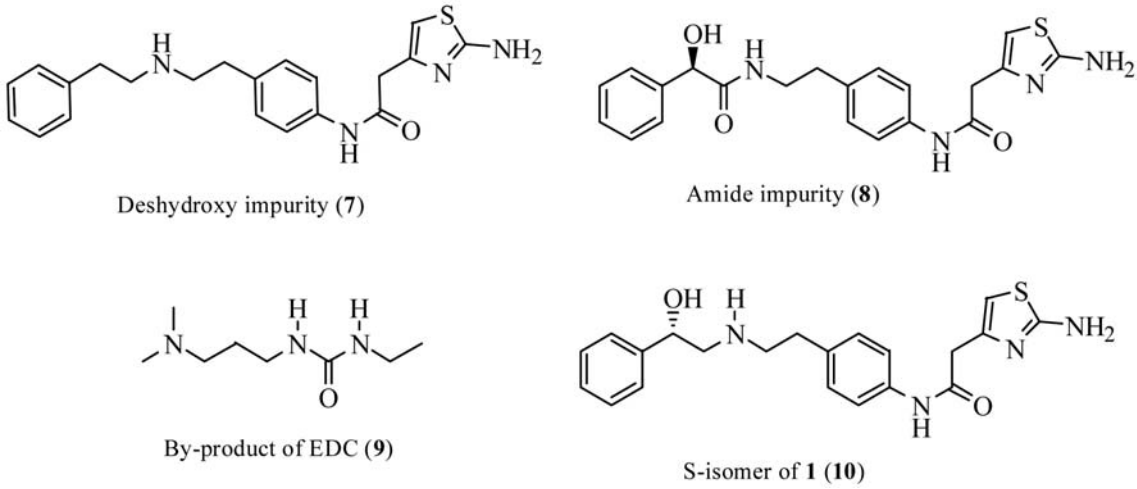


Figure 1. Process related impurities.

run of pilot scale, glass line reactors having variable rate agitation, with –10 to 150 °C jacket temperature range were used for the reaction. ^{1}H NMR spectra was recorded in DMSO-d_6 using Varian Gemini 400 MHz FT NMR spectrometer; the chemical shifts are reported in δ ppm relative to TMS. ESI mass spectra were performed on the Shimatzu LCMS-2020 spectrometer. Related substance purity was monitored by high performance liquid chromatography (HPLC) on Agilent Technologies 1200 series. The gas chromatography on Agilent Technologies 7683B with head space was used for analyzing the residual solvents.

5. 2. HPLC Method for Calculating the Chemical, Assay and Chiral Purity

Related substances, assay and chiral purity of Mirabegron (**1**) were estimated by a gradient HPLC analysis method developed at Megafine.

(a) Related substances and assay of Mirabegron (**1**) were estimated by using Zorbax SB-C8, (150 × 4.6 mm ID), 3.5µ column; mobile phase-A comprising a mixture of phosphate buffer (3.4 g potassium dihydrogenorthophosphate, in 1000 mL of HPLC grade water sonicate to dissolve, adjust the pH 6.8 with triethylamine and filter through 0.45 µm nylon filter and degas. Mobile phase-B comprising a mixture of acetonitrile/methanol/water in the ratio 45:45:10 (v/v/v); gradient elution: time (min)/A (v/v): B (v/v), $T_{0.0}$/85:15, $T_{8.0}$/85:15, $T_{35.0}$/70:30, $T_{45.0}$/70:30, $T_{48.0}$/85:15, $T_{55.0}$/85:15; flow rate 1.0 mL/min column temperature 35 °C, wavelength 245 nm. The observed retention time of Mirabegron under these chromatographic conditions is about 17.5 min.

(b) Chiral purity was estimated using Chiralpak IA (250 × 4.6 mm ID), 5µ column; mobile phase comprising a mixture of *n*-hexane, 2-propanol, methanol, THF and ethanolamine in the ratio of 183:67:67:17:1 (v/v/v/v/v) respectively; flow rate 1.0 mL/min.; column temperature 25 °C; wavelength 245 nm. The observed retention time of (*S*)-isomer is about 14.4 min and (*R*)-isomer (**1**) is about 16.8 min.

(c) Samples generated as described in the solid form were typically analyzed by X-ray powder diffractogram (XRPD). XRPD was conducted on a Bruker D8 advance X-ray powder diffractometer using Cu Kα radiation at 1.54. The instrument was equipped with a fine focus X-ray tube. The voltage and amperage of X-ray generator were set at 40 kV and 80 mA, respectively. The divergence slices were set at 0.3°. The diffracted radiation was detected by a Lynx Eye detector. Typically, a theta-two theta continuous scan at 4.95°/min (0.4 sec/0.033° step) from 2° 2θ to 50° 2θ was used. A corundum probe standard was used to check the peak position. In general, positions of XRPD peaks are expected to individually vary on a measurement-by-measurement basis by about ± 0.2° 2θ.

5. 3 Scale-up procedure for the synthesis of 2-(2-amino-1,3-thiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl] amino}ethyl)phenyl]acetamide (1)

Water (17.25 L), aniline **6** (1.15 kg, 3.93 mol) and acid **3** (0.63 kg, 4.01 mol) was added to the reactor. To the reaction mixture 37% HCl (0.409 kg, 3.93 mol) was added at 25–30 °C and stirred for 10–15 min. EDCI (0.828 kg, 4.32 mol) was added to the reaction mixture at 25–30°C and stirred for 2 h, completion of the reaction was monitored by HPLC. Upon completion of the reaction, *n*-butanol (11.5 L) and 20% ammonium hydroxide solution (0.92 L) was added to reaction mass and stirred for 25–30 min. Organic layer was preserved and aqueous layer was extracted with *n*-butanol (2.3 L). Combined organic layer was washed with 5% ammonium hydroxide solution (2 × 11.5 L) to eliminate acid **3**. Organic layer was then washed with 0.3% (4 × 17.2 L) brine solution. Organic layer was concentrated to about 40% of its initial volume to obtain turbid mass. To the concentrated mass, toluene (16.1 L) was added at 65–70 °C, cooled to 20 °C to obtain thick slurry and stirred at 20 °C for 2–3 h. Solid was filtered, washed with toluene (1.15 L) and dried in a vacuum tray dryer at 50 ± 5 °C for 2–3 h to provide 1.28 kg of **1** (84% yield). Purity by HPLC: 99.79%; *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}N_4O_2S$: 396.51; found: 397. ^{1}H NMR (DMSO-d_6): δ 1.60 (br s, 1H), 2.61–2.68 (m, 4H), 2.69–2.77 (m, 2H), 3.44 (s, 2H), 4.57–4.61 (m, 1H), 5.21–5.22 (d, 1H), 6.29 (s, 1H), 6.90 (br s, 2H), 7.10–7.12 (d, 2H), 7.19–7.23 (m, 1H), 7.27–7.32 (m, 4H), 7.48–7.50 (d, 2H), 9.99 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 168.29, 167.82, 145.87, 144.59, 137.17, 135.13, 128.77, 127.90, 126.74, 125.86, 119.04, 102.61, 71.51, 57.56, 50.77, 39.90, 35.40. Anal. Calcd (%) for $C_{21}H_{24}N_4O_2S$ (396.51): C, 63.5; H, 6.1; N, 14.1; S, 8.1. Found (%): C, 63.6; H, 5.82; N, 14.28; S, 7.96.

6. Acknowledgment

Authors thank the management of Megafine Pharma (P) Ltd. for permission to publish this work. Authors also thank colleagues of the Analytical Research and Development team for valuable inputs and support for this work.

7. References

1. M. R. Abu Bakar, Z. K. Nagy, A. N. Saleemi, C. D. Rielly, *Crystal Growth and Design* **2009**, *9*, 1378–1384. https://doi.org/10.1021/cg800595v
2. D. Z. Shende, Reactive crystallization in pharma industry International conference and exhibition on Pharmcognosy, Phytochemistry and Natural Products, October 21-23, 2013, Hyderabad, India.

https://www.omicsonline.org/proceedings/reactive-crystallization-in-pharma-industry-13978.html, (assessed: Dec 01, 2016)
3. J. Gras, *Drugs Today* **2012**, *48* (1), 25–32. https://doi.org/10.1358/dot.2012.48.1.1738056
4. T. Yamanishi, C. R. Chapple, K. Yasuda, K. Yoshida, R. Chess-Williams, *Neurourology and urodynamics* **2003***, 22*, 338–342. https://doi.org/10.1002/nau.10130
5. European Medicines Agency, assessment report (EMA/706651/2012), Assessed on Oct, 18, **2012**.
6. V. Khullar, G. Amarenco, J. C. Angulo, J. Cambronero, K. Hoye, I. Milsom, P. Radziszewski, T. Rechberger, P. Boerrigter, T. Drogendijk, EAU 2012 Poster AM12-2389.
7. P. Tyagi, V. Tyagi, M. Chancellor, *Expert Opin. On Drug Safety,* **2011,** *10.2,* 287–294. https://doi.org/10.1517/14740338.2011.542146
8. New Class of Treatment for Overactive Bladder Approved in Europe, Astellas Pharma Europe Ltd., Chertsey, UK, 11th January 2013. https://www.astellas.com/en/corporate/news/pdf/131111_eg.pdf
9. S. Kawazoe, K. Sakamoto, Y. Awamura, T. Maruyama, T. Suzuki, K. Onda, T. Takasu, US Patent Number 7,342,117, date of patent Mar 11, **2008**.
10. T. Maruyama, T. Suzuki, K. Onda, M. Hayakawa, H. Moritomo, T. Kimizuka, T. Matsui, US Patent Number 6,346,532, date of patent Feb 12, **2002**.
11. V. Peddy, R. Boge, PCT Int. Appl. 0156998, US Patent Number 9,283,210, date of patent Mar 15, **2016**.
12. ICH Quality Guideline, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html (assessed: November 18, 2016)

Povzetek

Razvili smo učinkovito metodo za neposredno izolacijo stabilne α-oblike kristalov mirabegrona (**1**), ki jo je mogoče uporabiti tudi z večjimi količinami. Razvita metoda zanika pretvorbo metastabilne β-oblike kristalov v α-obliko in s tem presega omejitve doslej objavljenih metod ter se s tem izogne dodatnim procesnim stopnjam med pripravo mirabegrona (**1**). Opisana metoda v eni stopnji neposredno daje stabilno α-obliko kristalov mirabegrona (**1**) z izkoristki okoli 84% in čistoto >99.77% (določene s HPLC).