Process Optimization and DOE Application in the Synthesis of Rociletinib Intermediate

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Critical step of Rociletinib synthesis, regioselective nucleophilic aromatic substitution of C-2 chlorine at pyrimidine molecule **3** with amine **2,** was optimised by implementation of pre-DoE screening and DoE-based algorithms. The synthesis reaction was developed and optimised by following a quality by design (QbD) approach, whereby a control strategy was developed for enhanced level of quality assurance. Optimisation of three main parameters (volume of solvent mixture, solvent ratio, and NaOAc quantity) resulted with good isomer ratio of over 8:1 with respect to desired C-2 isomer **4** with minimum of di-substituted impurity **6**. The crude product was, however, contaminated with Zn-based inorganics and had to be recrystallized to achieve acceptable purity and assay. Interaction of process variables and their corresponding effects on the percent conversion of maleic acid were discussed. Consistency of statistical model was verified by analysis of variance (ANOVA). API quality specifications (purity, impurity levels) are described across the modelling of process space, which was defined through quality target specifications (conversion NLT 70 %; Product Compound **4** NLT 50 %, Impurity **5** NMT 20 %, and Impurity **6** NMT 0.5).

Keywords:
organic synthesis, Quality by Design (QbD), statistical DoE

Introduction

As it is known, 5-fluoromethylpyrimidine derivatives are often used as key raw materials in the development of pharmaceutical products, mainly as various kinase inhibitors.[1–3]

Pyrimidine nucleosides containing a trifluoromethyl group at 5-position are known to have significant biological activity.[4] Fluorinated nucleosides and their analogues represent the class of organofluorine compounds, which found an extensive application in the 70's in biological chemistry, life-science, and medicine branches.[5,6] The anti-tumor activity of 5-fluorouracil and 5-trifluoromethyluracil derivatives is also reported.[7–9]

Capecitabine (Xeloda1) is the first FDA-approved oral chemotherapy drug for the treatment of both metastatic breast cancer and colorectal cancer, two of the most frequent solid malignancies worldwide. Capecitabine is now extensively prescribed in clinical oncology. This fluoropyrimidine has been designed as a less toxic and more specific alternative to canonical 5-Fluorouracil (5-FU).[10]

Rociletinib 1 is a novel, oral, targeted covalent (irreversible) mutant-selective inhibitor of the cancer-causing mutant forms of epidermal growth factor receptor (EGFR). It was designed to selectively target both the initial activating EGFR mutations and the dominant acquired T790M resistance mutation. Rociletinib is a new chemical entity that contains 5-trifluoro-2,4-diaminopyrimidine moiety as a critical structure motif (Fig. 1a).

Pyrimidines are known to be attacked by nucleophiles at positions C-2 and C-4 of pyrimidine ring. Many examples for nucleophilic substitution reactions of 2,4-dichloropyrimidine having a variety of substituents at 5-position (e. g. F, Cl, Br, I, CH_3, CH_3O, CN) with amines and alcohols, are known to result preferentially with 4-substituted aminopyrimidines. Interestingly, 5-trifluoro substituted pyrimidines provide almost 1:1 mixture of 2-amino and 4-amino product. The primary factors that influence selectivity of this addition are electronically negative nature of trifluoromethyl group accompanied by steric bulkiness. Triflurometyl group is blocking the approach of a negatively charged nucleophile to the sterically hindered 4-position.[11–14] As previously reported, addition of Lewis acids (e. g. $ZnCl_2$) improved the regioselectivity in favour of substitution at the 2-postion.[11,15–17] This reaction is described in several patents and articles, published by different authors.[11,18–21] The theory is that Zn complexes with the pyrimidine *N*-3 nitrogen, thus

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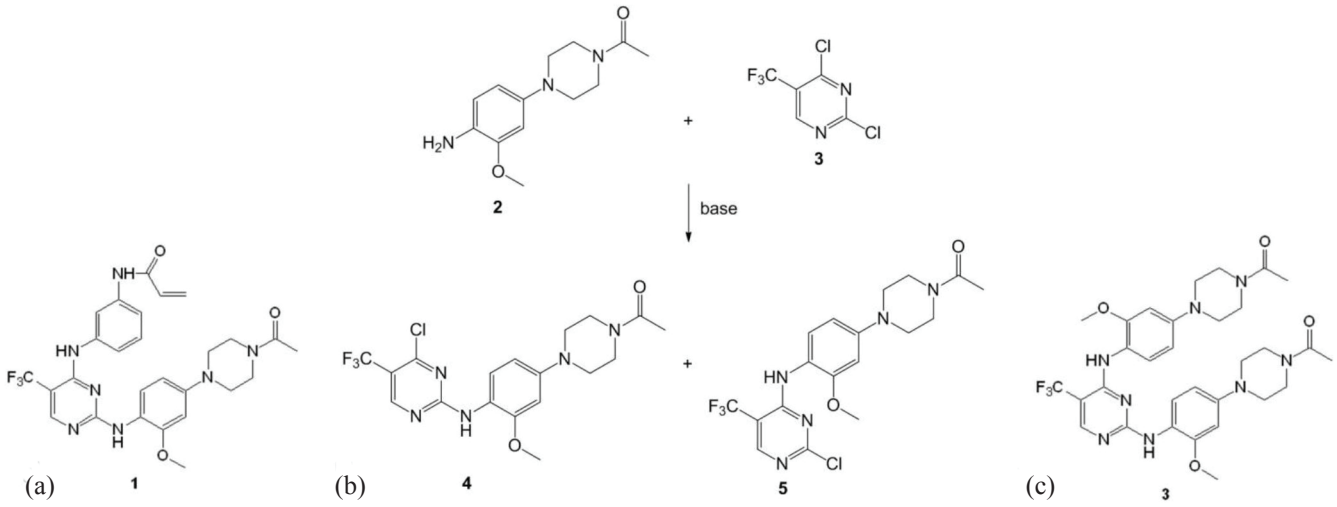

Fig. 1 – *Chemical structure of: a) Rociletinib **1**, b) regioselective nucleophilic aromatic substitution of C-2 chlorine at pyrimidine molecule **3** with **2** to give an amine **4** as major product and amine **5** as minor product, and c) di-substituted impurity **6***

directing chemistry to the less hindered site, to C-2. In this way, with appropriate solvent(s) and base, one could gain high isomer ratio with respect to desired C-2 isomer. Richter *et al.* from Pfizer presented a good review on this topic.[11] They tested a series of Lewis acids, and $ZnCl_2$ gave the most selective result. In the mixture of DCE/*t*-BuOH and Et_3N as a base (*t*-BuOH seemed to be quite important for regioselectivity), 10:1 isomer ratio (in favour of C-2 isomer) was obtained. Although Lewis acid should be present in catalytic amount, *o*-substituted anilines, particularly *o*-methoxy group at anilines (such as in compound 2) are involved in a chelating effect with the Lewis acid, so $ZnCl_2$ should be present even with 2 mol Eqs to obtain good selectivity. Even then, the reaction takes 24 hours to reach completion compared to typical 2–4 hours with anilines while selectivity is usually 5:1. Crystallization can be obtained due to moderate selectivity. Thus, firstly, $ZnCl_2$ must form a complex with pyrimidine **3** and then with *o*-methoxy substituted aniline **2**. Base should be involved to gain satisfactory conversion and regioselectivity (and added last, if possible).

A series of initial experiments have been performed, reaction conditions, solvents and bases have been tested (with or without the presence of $ZnCl_2$) to achieve the best isomer ratio: the best initial result was obtained with K_2CO_3 as a base, in the presence of $ZnCl_2$ in combination of CPME and *t*-BuOH as solvents – ratio 14:1 in favour of C-2 isomer was obtained, although the reaction was not completed.

In the development and optimization of this step there were several challenges; one of them was analytics. Following reaction kinetics was difficult because compound **3** was unstable in analytical conditions, and isomer compound **5** was reacting in the vial with compound **2** giving di-substituted compound **6** as an impurity (Fig. 1c). Therefore, reaction was usually carried out overnight (min. 20 h) and analysed afterwards.

The other major challenge was isolating pure compound **4** from the reaction mixture. Reaction takes place in the mixture of solvents in which compound **2** and compound **4** are not soluble, so isolation by extraction was not an option. One of the solvents was *t*-BuOH, which crystallizes at lower temperatures or *in vacuo*, so filtration in its presence was also not viable – although some attempts of filtration were done after the removal of *t*-BuOH by distillation. Such filtered crude cake consisted of compound **4**, its impurities (mostly **5** and **6**) and lots of inorganics – preferably $ZnCl_2$ and its hydrolytic products – and it had to be purified in the presence of water in which all the inorganics would be dissolved.

The application of Design of Experiments (DoE) approach to chemical development has been successfully used for several years.[22] More recently, the practice has been acknowledged by regulatory agencies within the concept of Quality by Design (QbD),[23] and it is becoming the base of any well-defined process development strategy within the industry. DoE Study is conducted with the purpose to estimate the effect of Critical Process Parameters (CPPs) and their interactions on relevant Critical Quality Attribute (CQA), and to determine optimum and robust conditions. CPP is a process parameter whose variability has an impact on a critical quality attribute (CQAs) and therefore should be monitored or controlled to ensure the process produces the desired quality.

Experimental

Materials

Amine **2** was produced in our laboratory by internal synthetic method. 5-CF_3-2,4-dichloropyrimidine **3** was supplied by Angene International Ltd. as high purity grade. All reagents and solvents were provided by Sigma-Aldrich or Merck, and were of reagent grade.

Analytical methods

Method for reaction monitoring and chromatographic purity: Instrument. Agilent 1260/1290 or equivalent Column & Packing: Acquity UPLC BEH C18, 2.1×100 mm, 1.7 μm Buffer: 10 mM KH_2PO_4 pH = 3.5 (pH adjusted with phosphoric acid) Mobile phase: A: Buffer, B: Acetonitrile gradient: 0. min – 95 % A, 20. min – 20 % A, 25. min – 10 % A, 25.01. min – 95 % A, 28. min – 95 % A Run time: 28 min Flow rate: 0.4 mL min^{-1} Injection volume: 1.0 μL Detector: 210 (BW 4 nm) Column temperature: 40 °C Autosampler temperature: 10 °C Diluent: acetonitrile:water 8:2. The process development in PLIVA was supported by Analytical Department, which is in accordance with highest pharmaceutical standards, such as GMP, GLP, etc. All analytical methods were developed and validated.

^1H (500 MHz) and ^{13}C (500 MHz) NMR spectra were recorded for pyridine solutions at room temperature using Bruker DRX500MHz spectrometer. Peak assignments were aided by ^1H-^1H COSY, HMBC and HSQC experiments.

Statistical methods (DoE, QbD)

Pre-DoE screening of all factors (parameters) on twolevels is conducted to determine CPPs and generally to reduce number of factors. Solvent choice is screened through principal component analysis (PCA). Range of process parameters is important (wide *vs*. narrow region), in process screening the wider range is used, while in late phase the narrower range in which product quality (defined by specifications of CQAs, for example impurities within control range) can be guaranteed. Ranges are also affected by downstream of the process (for example purification factor of isolation to meet specifications, etc.). DoE characterization of reduced number of factors on three levels is conducted to optimize reaction conditions in a systematic way to gain information about linear, interaction, and curvilinear effects. In general, QbD supports product robustness evaluations, product enhancement, as well as product troubleshooting. Pre-DoE as a screening, and QbD is supported through in depth knowledge of process.

Synthesis

Final procedure for preparation of 1-(4-(4-((4-chloro-5-(trifluoromethyl)pyrimidin- 2-yl) amino)-3-methoxyphenyl) piperazin-1-yl)ethanone (5)

A one-litre reactor was thermostated to 20–25 °C, and *t*-BuOH (277.5 mL) and $ZnCl_2$ (37.70 g, 0.277 mol) were charged. MTBE (277.5 mL) was added and reaction mixture was stirred at 20–25 °C for 1 hour (until colourless solution was obtained). Compound **3** (19.57 mL, 0.145 mol) was added and mixture was stirred at 20–25 °C for 1 hour to allow compound **3** to form a complex with zinc. Powdered NaOAc (11.34 g, 0.138 mol) was added and reaction mixture was stirred for one more hour at 20–25 °C. Finally, compound **2** (34.47 g, 0.138 mol) was added and reaction mixture (yellow suspension) was stirred at 20–25 °C for 20 hours. Upon cooling to 0 °C, water (300 mL) was slowly added dropwise to maintain the temperature at 0–5 °C (thus preventing hydrolysis of pyrimidine chlorine). After all water was added, MTBE and *t*-BuOH were distilled off at diminished pressure (bath temperature max. 45 °C) and acetonitrile (300 mL) was added into the remaining suspension. The yellow suspension obtained had a pH value of 3.5 and it was adjusted to 5 with 25 % ammonium hydroxide solution. Reaction mixture was warmed up to 70 °C when it dissolved. It was slowly cooled to 0 °C to initiate crystallization (seeding optional). The resulting suspension was stirred for 18 hours at 0 °C, filtered off and the solid was washed with a cold mixture of acetonitrile/water 1:1 cold mixture (2×120 mL) and dried at 65 °C/150 mbar/16 h yielding 51.33 g (86 %) of crude compound **5** (purity by UPLC: 95.13 area %, assay: 64.6 %). Crude compound **5** was purified by recrystallization as follows: 5 (45 g, 0.105 mol) was suspended in 450 mL ACN/water 1:1 mixture and warmed up to reflux temperature while stirring. Hot mixture (thin suspension) was filtered to remove traces of inorganics and the filtrate was warmed up again to 75 °C. Obtained solution was stirred at 75 °C for 20 minutes and cooled to 45–50 °C to initiate crystallization. It was stirred at 45–50 °C for 1 hour, then cooled to 20–25 °C and stirred overnight, cooled to 0 °C, stirred for 2 more hours and filtered off. Solid was washed with 45 mL ACN/water 1:1 cold mixture and dried at 65 °C/150 mbar/16 h to yield 29.25 g (65 %) of crystalline compound **5** (purity by UPLC: 99.65 area %, assay: 100.2 %).

Results and discussion

Pre-DoE screening

Screening of Lewis acids and content of $ZnCl_2$

Solubility of Zn compounds depends on the temperature and pH of the water in question. For

example, at pH<5, $ZnCl_2$ is stable. At neutral pH, Zn^{2+} in water is insoluble because it converts to $Zn(OH)_2$. Solubility increases with increasing acidity. Above pH 11, solubility also increases. Zinc dissolves in water as $ZnOH^+$(aq) or Zn^{2+}(aq). Examples of solubility of zinc compounds are: $ZnCl_2$ 4320 g L^{-1} and ZnO or $ZnSO_4 \cdot 7H_2O$ 580 g L^{-1}etc.[24,25] Unfortunately, anionic $ZnCO_3$ generated from K_2CO_3 has a solubility of 0.21 g L^{-1}– and when K_2CO_3 was used as a base – depending on its quantity and pH of the reaction mixture, we often ended up with huge amounts of insoluble $ZnCO_3$ which we could not remove until the final synthesis step. All intermediates and final Rociletinib have very low solubility and are hard to purify from inorganics. Therefore, we had to change the base to one which would (in case of significant change of pH) form a water-soluble Zn salt. Besides $ZnCl_2$, which gave satisfactory results regarding regioselectivity, LiCl resulted in the opposite selectivity, while $MgBr_2$ did not form a complex with compound **3** at all.

As mentioned previously, although Lewis acid should be present in catalytic amount, *o*-methoxy group at compound **2** is also involved in a chelating effect with the Lewis acid, so $ZnCl_2$ should be present with 2 mol Eqs to obtain good selectivity. For confirmation, reactions with no $ZnCl_2$ added resulted in low selectivity, even in benefit to C-4 isomer (in acidic conditions, main product is di-substituted pyrimidine **6**), and 1.2 mol Eq of $ZnCl_2$ was still not enough to gain a good selectivity.

Screening of solvents

Initial screening of solvents gave different results (Table 1). The main goal was to achieve good conversion, acceptable isomer ratio of C-4 and C-2 isomers and minimizing impurities as well. In the attempt to find the right reaction, solvent principal vector method was applied. A principal component analysis (PCA) is a way to picture the structure of the data as completely as possible by using as few variables as possible, and for that purpose statistical software is used (JMP 12.0). More than 120 different solvents and their combinations were searched according to their physical characteristics, *i.e.* melting points, boiling points, dielectric constants, refractive indexes, empirical polarity parameters, densities, lipophilicities and solubilities in water. The eigenvalues represent a partition of the total variation in the multivariate sample. Most important parameters were related with polarity and lipophilicity (with eigenvalues of 3.11 and 2.85 which represents 66.25 % of cumulative percentage of the behaviour explained for the eigenvectors). Non-polarity and non-lipophilicity of solvent was desired.

Also, pseudo components for mixtures (MTBE/*t*-BuOH = 1/1) based on molar ratio were used. PCA statistical analysis was performed and all ascending candidates were suggested. The main problems with group of candidates were: their reactivity (amines such as morpholine, TEA), high melting point (dioxane), low boiling point (diethylether), and safety (diisopropylether, benzene). Table 1 presents final results of statistical analysis of solvents and combinations of solvents which would give the best C-4:C-2 isomer ratio. Following the results shown above, an extended experimental research was applied to find the right solvent or combination of solvents. Selection of results obtained using different solvents and their mixtures in various volume ratios are presented in Table 2. The best obtained results were in 1/1 mixtures of CPME (cyclopentylmethyl ether)/*t*-BuOH and MTBE/*t*-BuOH. The final choice was MTBE/*t*-BuOH due to the lower boiling point of MTBE (easy to remove if/when needed) and its lower price. Although a huge number of experiments have been performed using CPME/*t*-BuOH in various ratios and volumes, from this point, mainly experiments with MTBE/*t*-BuOH will be discussed.

Table 1 – *Results of different solvents used for principal component analysis (PCA) by JMP*

Solvent	Predicted C-4:C-2 isomer ratio
CPME/*t*-BuOH 1:1	10
MTBE/*t*-BuOH 1:1	10
MeOAc	7
2-MeTHF	6
t–BuOH	4.5
dimethoxyethane	5
acetone	3.8
ethylacetate	3.4
HMP	3
diacetone alcohol	2.5
THF	2.4
2-propanol	2.5
2-butanol	2
acetonitrile	1.3
dichloromethane	1
ethanol, absolute	1
i-BuOAc	1
toluene	0.71
DMA	0.67
MeOH	0.63
NMP	0.40
MTBE	0.40

Table 2 – *Experimental pre-DoE screening of candidate solvents*

Entry	Solvent (V m^{-1})	Base (mol Eq)	Conversion after 20 h (UPLC, area %)	Isomer ratio 4:5:6
1	THF, 40 V	Et_3N 1.20	89.74	47.51 : 38.04 : hidden
2	DMA, 50 V	NMM 1.10	94.67	30.37 : 52.46 : 1.84
3	ACN, 50 V	K_2CO_3 1.10	70.65	41.37 : 21.98 : 0.38
4	DCM, 50 V	K_2CO_3 1.10	83.37	64.13 : 5.07 : 0.98
5	2-PrOH, 50 V	K_2CO_3 1.10	94.85	63.94 : 19.34 : 1.38
6	CPME, 50 V	Et_3N 1.10	92.23	63.50 : 25.86 : hidden
7	MTBE, 20 V	K_2CO_3 1.10	52.17	39.09 : 2.21 : 0.53
8	*t*-BuOH, 20 V	K_2CO_3 1.10	64.85	38.85 : 5.62 : 5.58
9	DME/*t*-BuOH 1:1.25 V	K_2CO_3 1.10	97.03	70.41 : 21.89 : 2.77
10	MTBE/EtOH 1:1.20 V	K_2CO_3 1.10	75.00	41.08 : 14.75 : 1.33
11	MTBE/2-PrOH 1:1.20 V	K_2CO_3 1.10	59.59	42.32 : 8.20 : 1.13
12	MTBE/*t*-BuOH 1:1.20 V	K_2CO_3 1.10	97.36	73.58 : 5.10 : 15.48
13	CPME/*t*-BuOH 1:1.15 V	K_2CO_3 1.10	95.55	74.78 : 6.46 : 4.32

Table 3 – *Experimental pre-DoE screening of bases (solvent CPME/t-BuOH, 50 V m^{-1}, $ZnCl_2$ 2.20 mol Eq)*

Entry	Base (mol Eq)	Conversion after 20 h (UPLC, area %)	Isomer ratio 4:5:6
1	K_2CO_3 1.10	86.52	58.02 : 4.10 : 0.83
2	Et_3N 1.10	99.24	46.26 : 40.84 : 5.48
3	DIPEA 1.10	86.07	69.74 : 7.02 : 2.29
4	NMM 1.10	41.73	8.51 : 4.62 : 0.22
5	NaH 1.10	74.35	48.17 : 7.38 : 0.71
6	*t*-BuOK 1.10	88.74	58.44 : 7.17 : 1.24
7	K_3PO_4 1.10	90.53	60.09 : 18.48 : 8.98
8	KOH 1.10	85.66	61.69 : 2.79 : 10.09
9	NaOAc 1.10	98.17	82.71 : 7.96 : 1.79
10	KOAc 1.05	81.41	63.61 : 7.37 : 2.34

Screening of bases

In Table 3, only results from CPME/*t*-BuOH 1/1 and MTBE/*t*-BuOH 1/1 mixtures are shown. The best choice of base regarding regioselectivity was the use of powdered K_2CO_3. Isomer ratio with respect to C-2 isomer was 14:1 although conversion was rather slow and reaction mixture had to be warmed up to 50 °C to achieve >90 % conversion. The second choice was *t*-BuOK, but it generated degradation impurities. All other bases gave lower isomer ratio (Table 3). Since the exclusion of K_2CO_3 due to generation of insoluble $ZnCO_3$, the focus became finding another viable base, which would generate soluble salts, such as NaOAc. Conversion is 95 % if reaction is carried out with NaOAc (1.05 mol Eq), in MTBE/*t*-BuOH 1/1 (16–20 V), at 25 °C for 20 hours. Besides, temperature increase results information of di-substituted impurity **6,** which is generated in the reaction between isomer **5** and amine **2** rather than between compound **4** and amine **2**.

Design of Experiments

From the pre-DoE experiments, it was concluded that three main parameters (volumes and ratios of MTBE/*t*-BuOH and mol Eqs of NaOAc) affect the overall product quality. The final screening design (JMP 12.0.) was chosen for factor screening. Table 4 shows the ranges of process parameters in main DoE characterization of three process parameters. The combination of continuous two-level categorical factors with central point replications was used to describe active two-factor interactions which exhibit strong curvature.

Volumes and ratio of MTBE/t-BuOH

Table 4 presents the results obtained using different volumes and ratios of MTBE/*t*-BuOH mixture. All experiments were done on 20-g batches using 2.00 mol Eq $ZnCl_2$.

Content of NaOAc and order of addition

Content of NaOAc varied from 1.00 to 1.50 mol Eq which was part of JMP12 design of experiments. Change in NaOAc content in the reaction mixture can be seen in Table 4.

Table 4 – *Final DoE with critical 3 parameters (Custom Design with minimal number of experiments)*

Entry	MTBE/*t*-BuOH (V m^{-1})	MTBE/*t*-BuOH ratio	NaOAc (mol Eq)	Conversion after 20 h (UPLC, area %)	Isomer ratio 4:5:6
1	10	1:1	1.50	96.14	70.00 : 9.83 : 1.54
2	10	5:1	1.00	89.92	28.22 : 25.59 : 0.28
3	18.5	1:1	1.05	94.34	75.73 : 9.02 : 0.61
4	20	1:1	1.05	89.61	66.25 : 10.57 : 0.28
5	20	3:1	1.25	71.19	46.30 : 25.70 : 0.19
6	30	1:1	1.00	89.89	69.93 : 7.62 : 0.61
7	30	5:1	1.50	25.00	13.91 : 4.13 : 0.00
8	20	1:1	0.00	46.56	30.41 : 2.92 : 0.82
9	18.5	1:1	1.05	95.53	76.29 : 8.28 : 1.89
10	18.5	1:1	1.05	95.38	77.17 : 8.52 : 2.33

The main conclusion is that excess NaOAc has no significant impact on the reaction profile, although hydrolytic impurities may increase. Conversely, the lack of base results in low conversion. Thus, 1 mol Eq of NaOAc is enough to complete the reaction, which is also confirmed by design of experiments (Fig. 2). NaOAc also has to be used in powdered form to enable the highest possible conversion. Different approaches regarding order of addition of reagents and starting compounds have also been tested. Anion generation at compound **2** followed by addition of previously prepared compound **3**/$ZnCl_2$ complex is not an option, since low isomer ratio is obtained in combination with slower con-

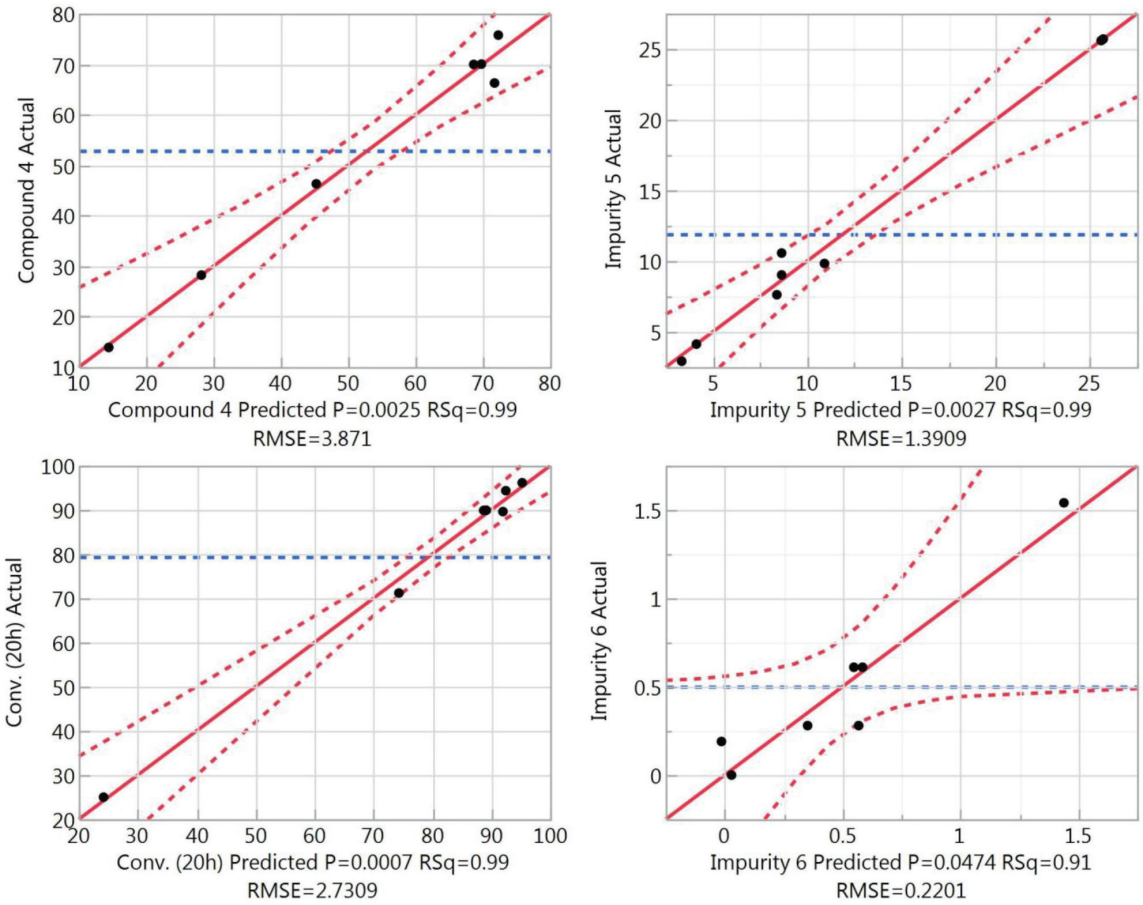

Fig. 2 – *Actual vs. predicted plot with summary of fit (RSq, R^2 and Root Mean Square Error, RMSE)*

version. In case when NaOAc was added 1 h after compound **2,** isomer ratio was lower (cca 6:1). The best isomer ratio was obtained when compound **2** was added 1 hour after addition of NaOAc to the complex; isomer compound **5** remained < 2 % and di-substituted compound **6** was cca 5 %. However, the material was heterogeneous, impure, and had to be recrystallized. Recrystallization was carried out in ACN/water 1/1 (20 V) in average 64 % yield giving overall yield of cca 54 %. The optimization of three parameters: volume of solvent, ratio of solvents, and content of NaOAc resulted in highest value of desirability – acceptable overall conversion, isomer ratio and content of di-substituted impurity (although isomer ratio was higher using higher volumes (Table 4, Raw 6)). The Standard Least Squares statistical model approach fits a wide spectrum of standard models that include regression, analysis of variance, analysis of covariance, and mixed models, as well as the models typically used

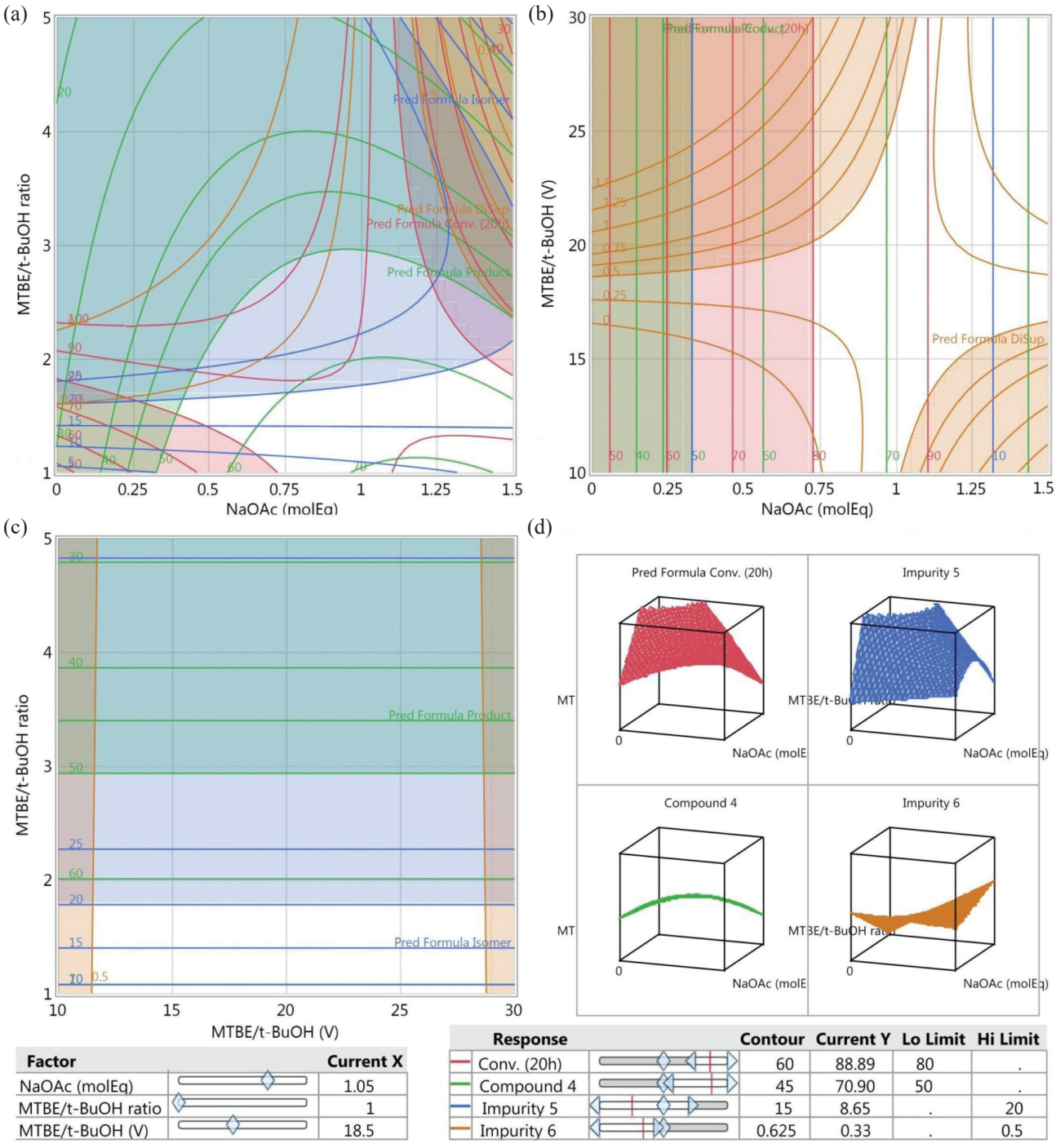

Fig. 3 – *Prediction profiler contour plot: a) RSA for mixture of MTBE/t-BuOH and mol Eq of NaOAc, b) volumes and mol Eq of NaOAc, c) mixture of MTBE/t-BuOH and mol Eq of NaOAc, and d) surface profiler*

to analyse designed experiments. In this paper, models are associated with experimental design and involve interaction and quadratic terms. To understand how each variable fit predicted the response, basic statistic is presented in Fig. 2. R^2 in all responses is > 0.97 (except in the case of Impurity 6 in which it is > 0.91).

In commercial process development, it is important to describe process parameter space in as little time as possible. For that purpose, custom design is used because of its flexibility. Process parameter investigation is made where the parameters were varied – main effects plus their interaction with one central point (default 12 experiments), but minimal number of experiments (Table 4 – 8 experiments) was used. Statistical power on individual parameters is between 0.4 – 0.6, which is significantly lower than 0.8 which is recommended for statistical analysis. Since the minimal number experiments are used there is some confounding between interactions of parameters. Replications (Table 4, Row 9, on 20-g scale batch, and Table 4, Row 10, on 35-g scale batch) were conducted to validate process optimal conditions and show reproducibility of methods used in this paper.

Analytic results are supported by compelling dynamic visualization tools, such as 2D contour plots profilers. These visual displays stimulate, complement, and support your understanding of the model. They enable optimisation of several responses simultaneously, and investigation of the effect of noise (Fig. 3). Prediction profiler with maximum desirability set for a Response Surface Analysis, RSA shows the result of the most desirable settings. Finding maximum desirability is an iterative process comprised of four different responses, two to maximize, Conversion and Product – Compound **4** and two to minimize, impurity **4** and **5**, and three factors (critical process parameters). Optimal reaction conditions calculated in JMP12 were 18.5 volumes of 1/1 mixture of MTBE/*t*-BuOH and 1.05 mol Eq of NaOAc. Fig. 3 shows the area (white surface) where optimum can be achieved: >80 % conversion, >50 % of product, Compound **4**, <12 % of C-4 isomer, impurity **5** and <1 % of di-substituted impurity **6**. The contour profiler is useful for viewing response surfaces graphically, especially when there are multiple responses as a part of QbD initiative. Model predictions were verified *via* results obtained in the laboratory by replications of optimal conditions, and the experiments showed good agreement with results obtained by statistical model.

In order to interpret the interaction of different process variables and their corresponding effect, contour plots were drawn (Fig. 3). The clear peaks of contour in Figs. 3a, 3b, 3c indicate that the optimum operating conditions are exactly within the design boundary (conversion NLT 70 %; Product Compound **4** NLT 50 %, Impurity **5** NMT 20 %, and Impurity **6** NMT 0.5). The combined effect of two independent variables on the response is well illustrated by two-dimensional response surface by maintaining remaining variable at fixed level.

Conclusions

– Pre-DoE screening of solvents by principal vector method was applied.

– Principal component analysis-most important parameters for good conversion and good regioselectivity were non-polarity and non-lipophilicity-chosen MTBE and *t*-BuOH combination.

– Screening of bases was performed experimentally and NaOAc was chosen as the most appropriate base regarding conversion, regioselectivity and simplicity of removal during isolation.

– The final design of experiments (JMP 12.0) was performed for process realized experimentally, thus confirming that the best conversion and regioselectivity was achieved by applying MTBE/*t*-BuOH solvent mixture in 1/1 volume ratio, 16–18 V m^{-1} and 1 mol Eq of NaOAc. Reaction was carried out by complexation of compound **3** (1.05 mol Eq) with $ZnCl_2$ (2.00 mol Eq), followed by addition of NaOAc, and finally addition of compound **2** (1.00 mol Eq) after one hour.

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