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Application of Quality by Design in the Synthesis of 6-substituted-2-aminobenzothiazole

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ABSTRACT

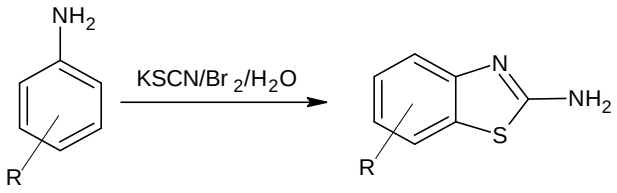
Background: 6-chloro-2-aminobenzothiazole is a key starting material for synthesis of many APIs. Literature reveals that synthesis of 6-chloro-2-aminobenzthiozole requires more time while giving poor yield. Therefore the purpose of this study was to achieve better yield & purity by application of QbD approach. **Methods:** The reaction was studied for effect of temperature and effect of light during the bromination and cyclization step. Each of the focus areas and process parameters were assessed for the likely risk they pose to the quality and yield (the CQAs) of the final product, 6-chloro-2-aminobenzothiazole. The reaction was optimized using Design of Experiment. Temperature and intensity of light were selected as the independent variables. The effect of the independent variables on the % yield, % purity and reaction time was studied. **Results:** The design space was developed between CPPs and CQAs of the focus area, i.e., Bromination, to facilitate the process of predicting the extent of effects of CPPs over CQAs. After a good understanding of the reaction mechanism, experimental and statistical evaluation of the results, it was found that temperature shows a significant effect on all the three CQAs whereas reaction time was governed by both temperature and intensity of light. **Conclusion:** The reaction was optimized using graphical and numerical evaluations and was found to best fit in terms of a reaction carried out in ice bath at 0°C and a 100W bulb. More scope for improvement was found in terms of application of DoE to the Recrystallization step.

KEYWORDS: 6-chloro-2-aminobenzothiazole, Bromine, QbD, temperature, intensity of light

1. INTRODUCTION:

Benzothiazole is a privileged bicyclic ring system consisting of a 5 membered 1,3-thiazole ring fused with a benzene ring. The nine atoms of the bicycle and the attached substituents are coplanar. This simple benzothiazole ring is present in most of the biologically active compounds. A lot of research is directed at evaluating New Chemical Entities (NCE), specifically benzothiazole derivatives, that possess interesting biological and pharmacological activities like- antimicrobial, antitubercular, antitumour, antimalarial, anticonvulsant, anthelmintic, analgesic and anti-inflammatory activites.[1-3] Due to these versatile chemotherapeutic activities, the molecule is of significant importance with wide pharmaceutical utilities. The synthesis of various aminobenzothiazole derivatives is of considerable interest. Moreover, substitution at 6 position of the aromatic benzothiazole ring is reported to be significant in increasing the pharmacological activity on account of its improving the lipophilicity of the NCE. The focus of discussion for this paper is 6-chloro-2-aminobenzothiazole which is the key intermediate compound formed in the synthesis of most clinically used drugs. The paper aims to study the factors effecting the synthesis of this lead compound.

The general method for the preparation of substituted-2-aminobenzothiazoles is described in two German patents.[4] The reported method by Matsui *et.al.* is a single step reaction, carried out between a substituted aniline and potassium thiocyanate followed by bromination in acidic condition at low temperature of 0-5°C. [5]



Scheme I

From literature it was found that though 2-aminobenzothiazole derivatives are easy to prepare and methods which use different catalysts or agents are available, these are associated with many limitations like costly starting materials, more number of steps being involved, harsh reaction conditions leading to generation of excessive impurities, etc.[6] Maintaining quality in the manufacturing of an API, begins early in the development stages when the chemist selects the

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synthetic route for manufacturing and evaluates the intermediate compounds for their quality attributes. The intermediate attributes impact the API specifications hence their understanding and control throughout the API manufacturing process leads to a safe, efficient and cost effective drug and subsequent drug product. [9]

There is still scope for more research work to be done in this field. In view of the importance of the benzothiazole nucleus containing compounds, in the present work attempt has been made to improve the synthesis reaction and remove the ambiguities associated with the earlier reported works. We have attempted to create a robust design space by applying scientific reasoning and understanding of all the material and process parameters to achieve better yield and purity of the product and at the same time reduce the reaction time. We envisioned to develop a method for direct synthesis of 6-substituted-2-amino benzothiazole under the holistic approach of Quality by Design (QbD). This is a scientific approach to design a product and process with performance characteristics which meet the specific predefined objectives. The three fundamentals and inter-related concepts of QbD approach are criticality, design space and control strategies. [10]

2. MATERIALS AND METHODS:

The experimental work was carried out by QbD approach in the following manner:

2.1 Step 1: Define critical quality attributes and perform risk assessment to propose design space:

The critical quality attributes (CQAs) of the final product (API) directly correlate to its potential impact of the safety and efficacy. For this purpose, before planning and establishing a design space, the CQAs for a drug substance/ API were determined and a prospective risk assessment of starting material and synthesis focus areas was executed in accordance with the ICH Q9 guidelines. [11]

2.2 Step 2: General procedure for synthesis:

Materials: Herein, we report a simplistic synthesis of a 2-amino benzothiazole derivative, 2-amino-6-chlorobenzothiazole, starting from cheap and readily available materials of laboratory and/or analytical grade.

The reaction under varying conditions of temperature and light. 6-chloro aniline (0.045 mol, 5.74 g) and potassium thiocyanate (0.077 mol, 7.5 g) were dissolved separately in concentrated acetic acid (quantity sufficient to dissolve) and kept for mixing in a 3 necked round bottom flask, fitted with a mechanical teflon stirrer rotating at 400 rpm. A solution of bromine (0.09 mol, 4.645 ml) in acetic acid was added slowly drop by drop from a separating funnel with continuous stirring of the reactants. The bromine addition was carried out for a period of 45 minutes. The reaction was monitored using Thin Layer Chromatography (TLC) technique on Silica Gel G TLC plates in a solvent system of chloroform:ethanol (90:10).

After bromine addition, stirring was continued for additional 2 hours in the specific temperature and light conditions. The yellow solid residue at the bottom was collected and treated with ammonia to form 2-amino-6-chlorobenzothiazole (crude product); while the liquid was filtered and neutralized with conc. ammonia solution to pH 7. The yellow precipitate was collected and mixed with the earlier fraction of crude product and recrystallized from ethanol, giving activated charcoal treatment, to yield pale yellow to white amorphous powder.

2.3 Step 3: Development of Experimental Design for optimization of reaction:

The prior knowledge and scientific understanding of chemistry and the manufacturing process derived from the risk assessment results influenced the scheming of design space. The optimization of the reaction was done by using the Design Expert® trial version 9.0.1.0 Software (State Inc, Minneapolis, MN). A Response Surface study type using User Defined Design with a =0.05 was employed. Based on Step 1 and Step 2 study results, Temperature and Intensity of light were selected as the independent factors, studied at three and two levels respectively. All other conditions and processing variables were kept invariant throughout the study. Table 1 summarizes an account of the 8 experimental runs studied, their factor combinations. The response variable evaluated were % Yield (Y_1), % Purity (Y_2) and Reaction time in minutes (Y_3).[12,13]

Table 1: Experimental Trials with factor combination

Trial No.	Temperature (C)	Intensity of light (Watts)
1	0-5	80
2	0-5	100
3	25	100
4	25	60
5	75	100
6	75	80
7	0-5	60
8	75	60

2.4 Step 4: Evaluation of experimental results:

2.4.1 Reaction time: The time when only a single spot of final product, 2-amino-6-chlorobenzothiazole, with no interference from 6-chloro aniline was seen on the TLC plate, was used to determine the reaction completion time. The spots were observed under UV cabinet.

1.4.2 Yield: The practical yield was determined after recrystallization of the crude product.

% Yield= (Practical Yield /Theoretical Yield) * 100

2.4.3 Characterization of the product:

2.4.3.1 Identification: The melting points of the synthesized compounds were determined using Thermionik Melting Point Boiling Point Apparatus in open capillary. The IR spectra of the synthesized compounds were recorded on a Fourier Transform Infra Red (FTIR) spectrometer (model Shimadzu 8700) in the range of 400-4000 cm^{-1} as KBr pellets.

2.4.3.2 Purity: Purity was determined on Perkin Elmer UV-200 model using a reported HPLC method, summarized in the Table 2.

Table 2: HPLC method for evaluation of purity

Parameters	Description
Column	Kromasil 100 °A C18. 4.6 mm x 100 mm x 5µm
Mobile Phase	Methanol : Water (60:40 v/v); pH maintained at 2.5 using conc. Acetic acid.
Temperature	Ambient
Flow Rate	1 ml/min
Detection	UV 254 nm

3. RESULTS:

3.1Risk Assessment Results: The Table 3 summarizes the quality attributes of assay and impurity levels as the ones critically impacting the safety and efficacy of the final product, while the other attributes were important to be ensured within the specifications but did not pose risk to safety and efficacy. Hence the primary focus for the experimental design was intended at establishing boundaries for the control of assay.

Table 3: CQAs for 2-amino-6-chlorobenzothiazole

Quality Attribute	Specification	Criticality	Justification
Description	Colour, Form, Odor	No	ICH Q6A, Universal test
Identification	FTIR	Yes	ICH Q6A, Universal test
Potency	**Assay**	**Yes**	**ICH Q6A, Universal test**
Purity	Related Substance		ICH Q6A, Universal test
	Genotoxic Impurities	No	No genotoxic impurity identified
	Residual Solvents	Yes	Class 2 solvents are used in manufacturing process
	Metal Residues	No	Non-metal cyclizing agent has been used in manufacturing process
	Heavy Metals	No	Pharmacopoeial Test
	Residue on ignition	No	Pharmacopoeial Test
Physico-chemical Properties	Melting Point	No	No polymorphs have been observed
	Solubility	No	
Polymorphism		No	ICH Q6 A. No polymorphs have been observed.
Particle Size		No	ICH Q6 A.
Optical Activity	Stereoisomerism	No	ICH Q6A. The drug substance is optically active substance with a chiral carbon center.
Water Content	LOD	No	The drug substance shows no hygroscopic property.
Microbial limit	Not Applicable	No	ICH Q6A. Drug substance is not capable of supporting microbial growth

The synthesis was tailored to each process step. The attributes at each step were assessed relative to their impact on relevant CQAs of the final product within that focus area (FA) of the manufacturing process. The list of FAs and the risk associated at that level was as found to be as shown in Table 4.

Table 4: List of risk prone focus areas of synthesis

Focus Area	Risk
Initial Mixing	Low
Cyclization Reaction	High
Final Mixing	Low
Filtration	Low
Recrystallization	High
Drying	Low

The main reaction step, comprising of cyclization of the two starting materials in presence of liquid bromine was found to pose high risk to CQAs of final product. The identification of factors impacting purity, yield and reaction time at these high risk prone FAs is summarized in the Table5.

Table 5: Initial Risk Assessment of Cyclization Reaction Step

Parameter	Risk	Justification
Material Variables:		
Molarity of starting material	Low	Cyclization reaction. Stoichiometry is not significantly important
Reaction Condition variables:		
Temperature	High	Bromine addition generates heat. Temperature affects the yield of the final product.
Light	High	Light increases the rate of Bromine (free radical) cyclization. It also shows effect on the purity of the final product. Light changes the efficiency of the reaction
Speed	Low	Kept constant at 400 rpm
pH	Medium	Acidic pH must be maintained for protonation.
Apparatus Variables:		
Stirrer material	Medium	Acidic pH is maintained. Brominating generates heat. Chemically resistant material required. Hence medium risk

The temperature and intensity of light effect the reaction and thus the Critical process parameters (CPPs) for the manufacturing of the final product. Good scientific rationale and prior knowledge of the complexity of this FA invigorated the experimental work. The reaction was conducted at different combinations of these two factors as described in the experimental section in steps 2 and 3.

The impact of these two factors was evaluated on the various qualities of the final product and are discussed in the following two subsections. This paper makes an exceptional attempt at studying the effect of the intensity of light on the classical bromine cyclization.

3.2 Physical and characterization results of 2-amino-6-chlorobenzothiazole:

The physical evaluation results of the experimental trials were as shown in Table 6. Sharp melting points were obtained.

Table 6: Physical Evaluation and Characterization Results of the Experimental Trials

Trial No.	Temp. (°C)	Intensity of light (Watt)	M.P. (°C)	% Yield (Y_1)	% Purity (Y_2)	Reaction Time (min) (Y_3)
1	0-5	80	204	67.55	61.002	50
2	0-5	100	203	22.37	98.17	48
3	25	100	201	85.34	58.052	42
4	25	60	204	68.007	47.023	45
5	75	100	-	0	0	0
6	75	80	-	0	0	0
7	0-5	60	201	52.67	61.643	50
8	75	60	202	0	0	0

3.3 Spectral studies of 2-amino-6-chlorobenzothiazole:

Mol. Formula: $C_7H_5ClN_2S$, Mol. Weight: 184.64, IR (KBr, cm^{-1}): 3088.07 (C-H aromatic), 1532.34 (C=C aromatic), 564.72 (C-S), 1633.82 (C=N), 1532.387 (C-C aromatic), 3457.84(NH), 811.722 (C-Cl).

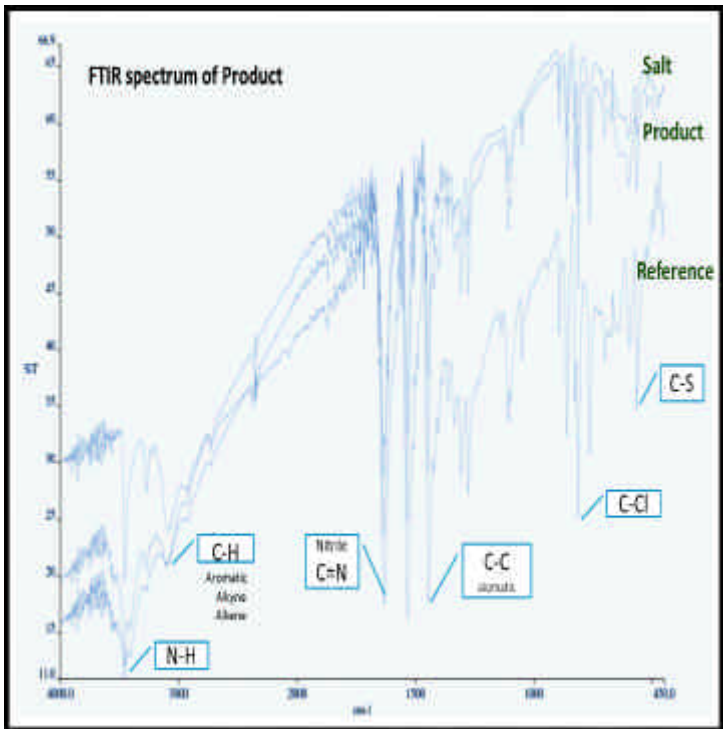


Figure 1: FTIR spectrum of 6-chloro-2-aminobenzothiazole

Similarly 4.0 (S, 2H) NH_2, 7.56-8.13 (3H, M) CH benzothiazole were observed in H^1NMR spectrum of the final product, thus identifying the product to be 6-chloro-2-aminobenzothiazole.

3.4 Mathematical modeling of RSM optimization:

From the statistical design of experiment (DoE) approach, the Design Expert Software gave the Analysis of Variance (ANOVA) output, checked at 5% significant level shown in Table 7. The software removed the insignificant terms by backward integration. The model was considered significant if the p-value (significance probability value) was less than 0.05.

Table 7: **ANOVA table for Response Surface Models**

Backward Elimination Regression with Alpha to Exit = 0.100

Source	Sum of Squares	df	Mean Square	F Value
% Yield (Y_1)				
Quadratic Model	7587.62	2	3793.81	15.67
A-A	*3388.65*	*1*	*3388.65*	*14.00*
A^2	*4198.96*	*1*	*4198.96*	*17.34*
% Purity (Y_2)				
Linear Model	8126.54	1	8126.54	36.45
A-A	*8126.54*	*1*	*8126.54*	*36.45*
Reaction Time (Y_3)				
Quadratic Model	4186.88	3	1395.63	1860.83
A-A	*3650.67*	*1*	*3650.67*	*4867.56*
B-B	*4.17*	*1*	*4.17*	*5.56*
A^2	*532.04*	*1*	*532.04*	*709.39*

Source	p-value Prob > F		Adj R Square	Pred R Square
% Yield (Y_1)				
Quadratic Model	0.0070	significant	0.8074	0.6606
A-A	*0.0134*			
A^2	*0.0088*			
% Purity (Y_2)				
Linear Model	0.0009	significant	0.8587	0.8351
A-A	*0.0009*			
Reaction Time (Y_3)				
Quadratic Model	< 0.0001	significant	0.9987	0.9963
A-A	*< 0.0001*			
B-B	*0.0779*			
A^2	*< 0.0001*			

From the above table, unexpected results were observed. The Main Effect of Temperature was found to be significant (P<0.05) in each response whereas the interaction between Temperature and Intensity of light was not seen and hence inferred to be statistically insignificant. Similarly the second factor of Intensity of Light showed significant main effect in the third response of Reaction Time (P value= 0.0799) alone.

Final Equation in Terms of Actual Factors: The regression equation showing the mathematical relationship, in the form of polynomial equations, between the main effects Temperature (A), Intensity of Light (B) on the dependent variables (responses) % yield, % purity and reaction time was as shown below.

$$\% \text{ Yield} = 47.53000 + 3.28208 * A - 0.084654 * A^2$$

$$\% \text{ Purity} = 77.53875 - 1.47210 * A$$

$$\text{Reaction Time} = 52.66667 + 0.52000 * A - 0.041667 * B - 0.030133 * A^2$$

The equation, in terms of actual factors, gave quantitative predictions about the response for any given levels of each factor (speci-

fied in the original units). But these equations could not be used to determine the relative impacts of each factor because the coefficients were scaled to accommodate the units of each factor while the intercept was found to be not at the center of the design space.

The scattered plot of predicted Vs actual values for each response is represented in the Figure 2. The plot (a) and (b) shows that the actual values of % Yield and % Purity do not fit their respective model as good as plot (c), Reaction Time. It is thus clear from the graphs that for yield and purity, there is a group of values, which are not easily predicted by the models. For (c) the points were found to be very close to the Fit Line, thus concluding that this model predicts the reaction time well, based on the chosen factors.[13-17]

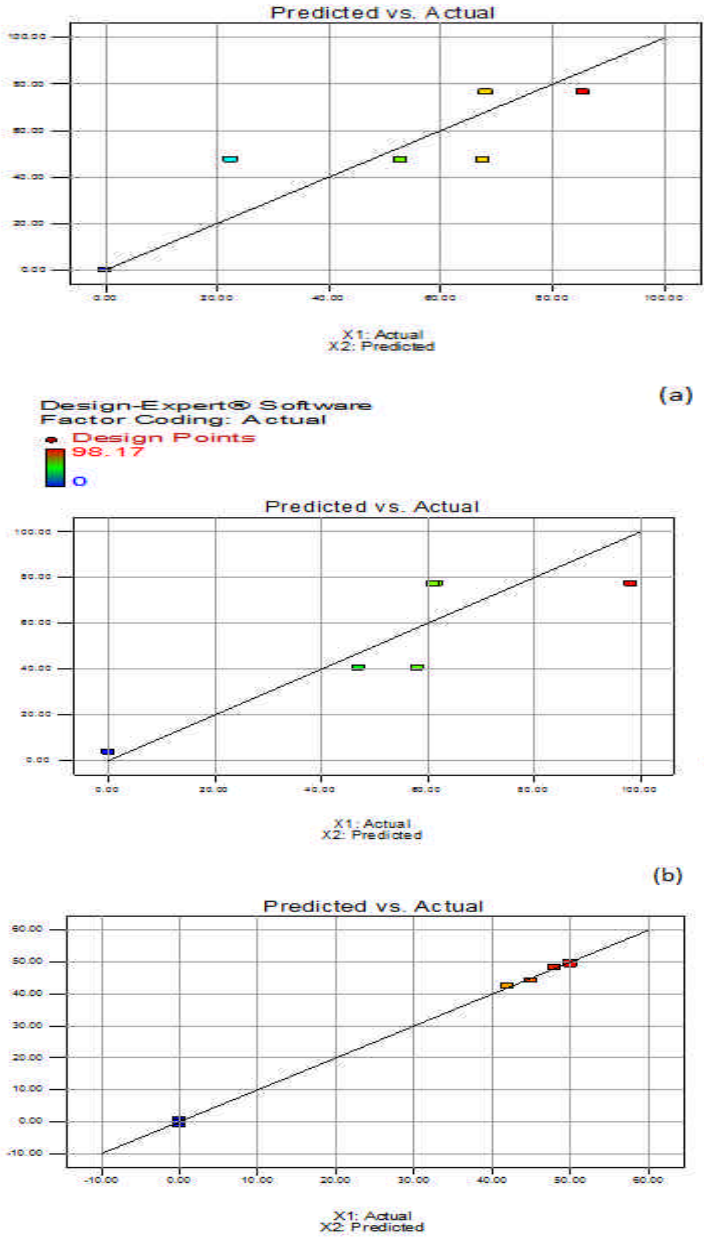
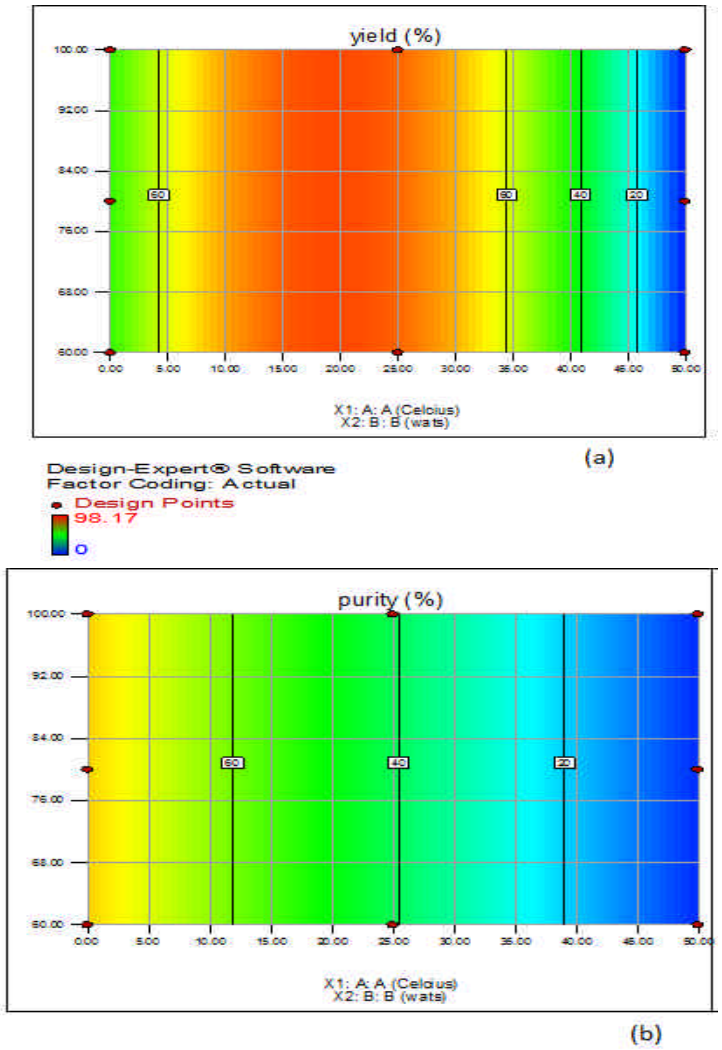


Figure 2: Actual Vs Predicted Values for (a) % yield, (b) % purity, (c) reaction time

Response Surface Analysis:

Figure 3 represents the two dimensional (2-D) contour plots, constructed based on the model polynomial functions, using Design Expert software. These plots showed unanticipated results of interaction effects of the factors on the responses. While 25°C and 100 W was found to give good yield, it also took the least time for completing the cyclization, in comparison to other conditions. But at such reaction conditions purity had to be compromised. The evidences of fast reaction completion in the presence of high intensity light, help to infer that the cyclization by Bromine is light driven. Though reaction seemed to be completed within one hour, complete closure of the ring requires time. Hence stirring is continued for another 2 hours.

The effect of Intensity of Light were found to be insignificant on yield and purity, as evident from the parallel black lines in Figure 3(a), (b). Temperature has comparatively greater influence on the response variables than Light. While in (c), Intensity of Light showed greater interaction with temperature in the range of 0^0C to 20^0C. From the contour plots it is evident that the reaction fails at high temperature conditions. Hence this condition was discarded from consideration. [18-20]



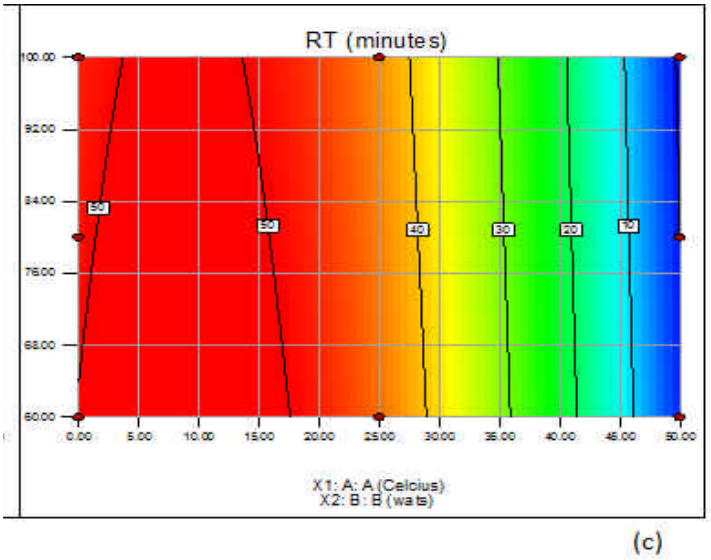


Figure 3: Contour plot showing the effect of Temperature and Intensity of Light on % Yield, % Purity and Reaction Time (RT)

Optimization of the independent variables of the reaction:

This was the most important part of response surface methodology. The combination of factors values which gave the desired response values was selected to be optimum for this reaction. The criteria or constrains for optimum reaction are given in Table 8.

Table 8: Constrains for optimization

Name	Goal	Lower Limit	Upper Limit
A:A	is in range	0	25
B:B	is in range	60	100
% Yield	maximize	0	85.34
% Purity	maximize	70	100
Reaction time	minimize	40	50

The software gave 10 solutions from numerical optimization, of which the highest desirability solution suggested the reaction carried out in an ice bath at 0°C under a 100 W intensity lamp shall be completed in 48.5 minutes and would give a product yield of 47% showing 77.5% purity. The whole cyclization and other associated work-up steps were found to complete within 4 hours.

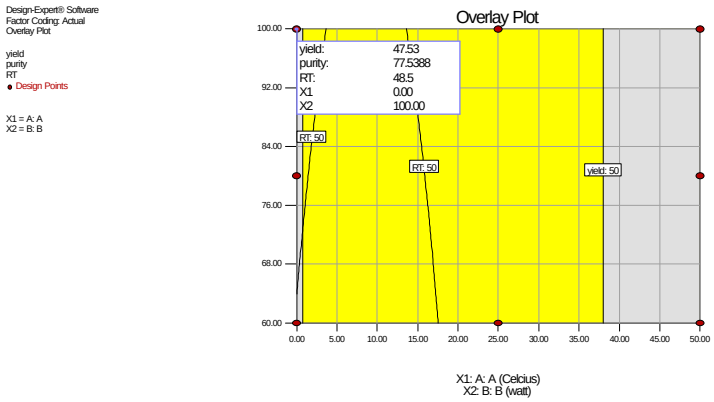


Figure 4: Overlay plot of the design space

Figure 4 is an overlay plot of yield and quality (relating to purity and assay), setting the foundation for continued improvement of the process. The yellow area is where the optimal conditions may be obtained under current manufacturing conditions. The grey area represents lower yield and tolerable quality but fast reaction completion time. However, new technology and process innovation may provide justification to explore this area in the future. The grey area is approaching the tentative limits of less than 80 % purity of design space.

DISCUSSION:

The reaction mechanism on thorough study, reveals that bromine allows in-situ cyclization of substituted aniline and potassium thiocyanate (KSCN) to give the desired product, 2-amino-6-substitutued-benzothiazole and its hydrobromide salt. An initial attack of aniline lone pair of electrons takes place on the electrophilic carbon center of KSCN, in the presence of conc. Acetic acid, which helps in protonation. The acidic environment allows formation of an intermediate (4-chlorophnyl)carbamimidothioate. In second step, bromination to ortho-position of the amine takes place; this allows subsequent in-situ cyclization to give desired product. The cyclization with bromine is basically achieved by oxidation of substituted aniline and alkali thiocyanate in the acidic environment.

The S and N nucleophilicity allows for hetero atom linkage between any points on the benzothiazole nucleus. The FTIR spectra of the product and the salt were found to completely superimpose, allowing to infer that the salt is not to be discarded but worked-up to get our desired product.

To get better optimization of this critical reaction the following alternatives can be employed to improve the design space:

By changing the constrains given to the software for optimization, we can achieve targeted response value of either % Yield or of quality (% Purity) for the 6-chloro-2-aminobenzothiazole, as per the requirement of the manufacturer. In any case, the manufacturer or scientist has to compromise on the other attribute.

The impurities in the final product can be identified and specific limits to these impurities can be adopted as per specifications. These can be kept under high scale monitoring from the early manufacturing process steps. High level impurities can be specifically scrutinized from the early stages to meet the down-streamed CQAs.

The issue related to the poor quality can be avoided by assessing and reducing the high risk disposed to Recrystallization step of the

manufacturing of final product. Ethanol was used as the solvent in this step. Another flexible design space should be created based on the knowledge acquired from the risk assessment. Design space boundaries should be established for each relevant variable in relation to every other variable for recrystallization process. Appropriate control strategy should be set for every variable which impacts purity levels of the product.

The work carries a future scope of extending the process for validation. This lab-scale design can be critically considered to check for its process scale-up sensitivity before implementing for large scale manufacturing.

CONCLUSION:

The small and simple benzothiazole nucleus containing molecules possess interesting biological activities. Literature reveals that synthesis of 6-chloro-2-aminobenzthiozole requires 2-3 steps and hence takes more time while giving poor yield. The protocol described for preparing 6-substituted-2-aminobenzthiazole derivative using liquid bromine as cyclizing agent, is efficient with the advantage of using QbD approach. The proposed method is feasible from industrial point of view because of less number of steps, commercially available reagents and easy to handle and reaction conditions. The synthesized product can be envisioned as an intermediate which can act as "control gates" for quality and/or economic risk to determine if the intermediate is of appropriate and adequate quality to proceed for further processing. In fact, use of this approach renders it highly improbable for the API CQAs to be adversely effected by process parameters in the earlier stages. Understanding and control of the intermediate QAs as they progress through the synthesis process will lead to identification and control of any additional CQAs and CPPs within the manufacturing process.

The design space provides assurance that when the at all focus areas, the process is maintained within the specified limits, then the product will meet the predetermined QAs. A significant intermediate, like 6-substituted-2-aminobenzothiazole, in the manufacture of APIs reduces risk associated with quality attributes of process to API CQAs. Unlike for drug product manufacturing, the drug substance manufacturing has intermediate compounds, which when monitored for quality and safety, reduce the overall risk which impact CQAs for the API.

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