American Journal of Analytical Chemistry, 2016, 7, 494-524
Published Online June 2016 in SciRes. http://www.scirp.org/journal/ajac
http://dx.doi.org/10.4236/ajac.2016.76047

QbD Approach Method Development for Estimation of Dabigatran Etexilate along with Its Impurities and Identification of Degradants in Capsule Dosage Form

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Received 7 April 2016; accepted 13 June 2016; published 16 June 2016

Abstract

The concept of Quality by Design was demonstrated in the development of a stability-indicating assay and related substances method by HPLC for Dabigatran Etexilate Capsules dosage form. Method design, method evaluation, method control and life cycle management were explained by systematic flow chart. Analytical Target Product profile was defined. The method was developed using the Inertsil ODS-3V, 150 mm × 4.6 mm, 5 µm column using the gradient program with ammonium formate buffer as mobile phase A and acetonitrile as mobile phase B. Risk assessment was performed as part of method evaluation. Design of experiment tools was used to optimize the chromatographic conditions. A two-level Full Factorial Design along with Face Centered Central Composite design augmentation was employed and statistical analysis of the experimental data uncovered the significant influential of chromatographic factors. The design space and the contour plot suggest that the current center point parameters can be further modified, resulting in better acceptability of the response parameters. The performance of the optimized method was validated according to current ICH guidelines. Dabigatran Etexilate Capsules was subjected to various stress conditions like oxidative, acid, base, hydrolytic, thermal, humidity, and photolytic degradations and evaluated chromatograms at 220 nm. The degradation products were well separated from each other and main peak, demonstrating the stability-indicating power of the method. One of the major degradant impurities, which are forming in neutral hydrolysis stress condition, is

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How to cite this paper: Bapatu, H.R., Maram, R.K., Cho, W.H. and Pasagadugula, V.B.R. (2016) QbD Approach Method Development for Estimation of Dabigatran Etexilate along with Its Impurities and Identification of Degradants in Capsule Dosage Form. *American Journal of Analytical Chemistry*, **7**, 494-524. http://dx.doi.org/10.4236/ajac.2016.76047

isolated and characterized by using analytical techniques like IR, LC-MS and NMR. Degradation pathway for Dabigatran Etexilate was proposed based on forced degradation data along with reaction mechanism.

Keywords

Quality by Design, Design of Experiment, Dabigatran Etexilate, Degradant Impurities

1. Introduction

Dabigatran Etexilate (DABE) is in a class of anticoagulant (or blood thinner) medications called direct thrombin inhibitors. It works by preventing blood clots from forming in the body. DABE is used to help prevent strokes or serious blood clots in people who have non-valvular atrial fibrillation. Atrial fibrillation is a condition where the heart beats irregularly, thereby increasing the chance of clots forming in the body, which can lead to a stroke [1] [2].

The double prodrug DABE is rapidly converted to active metabolite dabigatran (DAB) by esterase catalyzed hydrolysis after dosing in humans. Microsomal incubation studies suggest that human carboxylesterases (CES) are likely to play an important role in the formation of the active moiety [3] [4].

The chemical name for dabigatran Etexilate Mesylate is *β*-Alanine, N-[[2-[[[4-[[[(hexyloxy)carbonyl]amino] iminomethyl] phenyl]amino]methyl]-1-methyl-1H-benzimidazol-5-yl]carbonyl]-N-2-pyridinyl ethyl ester, methanesulfonate. DABE is a yellow-white to yellow powder. A saturated solution in pure water has a solubility of 1.8 mg/mL. It is freely soluble in methanol, slightly soluble in ethanol, and sparingly soluble in isopropanol. It is available in 75 mg, 110 mg and 150 mg capsule dosage form. The 150 mg capsule for oral administration contains 172.95 mg DABE Mesylate, which is equivalent to 150 mg of DABE.

The structures and chemical names of DABE Mesylate and its impurities are presented in **Figure 1(a)** & **Figure 1(b)**. Pharmacopeia monographs are not available. Analytical methods for the estimation of DABE Mesylate were reported by HPLC attached with UV/PDA detectors and also with other detectors like mass and aerosol but not much on the degradation products [5]-[10].

HPLC method for the analysis of DABE Mesylate and its known ten impurities supported by Quality-by-Design [11] was published, but not reported about degradation products which were forming in forced degradation study. A validated HPLC method for the estimation of DABE dug substance and its impurities was reported [12], which had 55 min of runtime and contains ion pair reagent. However, impurity B and impurity H which were specific to DABE Mesylate drug substance manufacturing process were not a part of any of these publications.

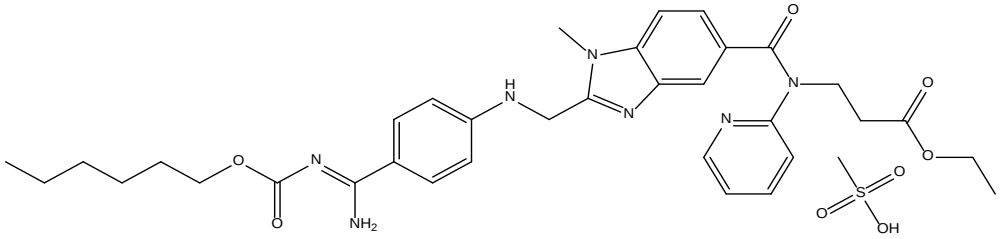
Hence the aim of this research is to develop stability indicating HPLC method for estimation of DABE Mesylate and its impurities which are part of manufacturing process and degradation in the capsule dosage form by using QbD principles.

Quality-by-Design (QbD) methodology is a very useful tool for the development of robust and reliable analytical methods [13]-[20]. Publications on the DoE and adopting QbD approach are available on internet to obtain the multidimensional robust design space [21] [22]. Experimental design and measurement system analysis are structured risk assessment tools for the evaluation of method robustness and ruggedness. Many authors adopted QbD tools and applied for method development [23]-[30].

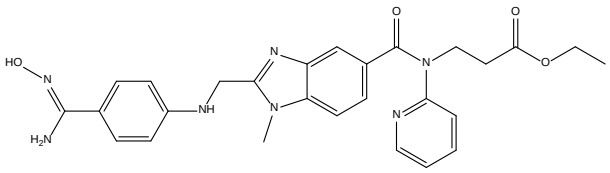
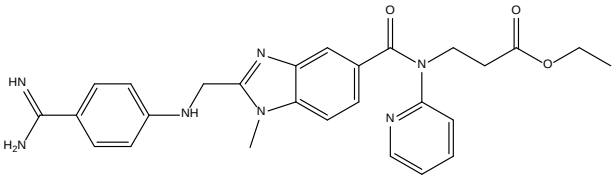
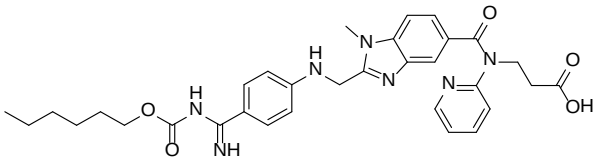
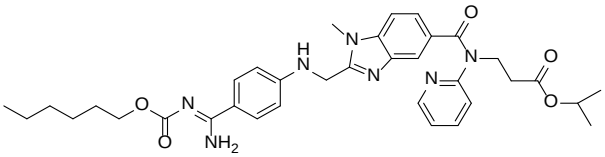
2. Experimental

2.1. Materials

PRADAXA (DABE Mesylate) Capsules received from Boehringer Ingelheim, India. DABE and its impurities are available from Simson Pharma, Mumbai, India. Acetonitrile (HPLC-grade from J.T. Baker, USA), Ammonium acetate, Ammonium formate Hydrochloric Acid, Hydrogen Peroxide, Sodium hydroxide were from Merck (Darmstadt, Germany). Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.45 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.



(a)

Impurity Name	Impurity structure	Remarks
Impurity-B Chemical Name: 3-[[2-[(4-(N-Hydroxycarbaimidoyl)-phenyl amino) methyl]-1-methyl-1H-benzoimidazole-5-carbonyl] pyridine-2-yl-amino]-propionicacid ethyl ester		Process related Impurity.
Impurity-C Chemical Name: ethyl 3-(2-(((4-carbamimidoylphenyl) amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo[d]imidazole-5-carboxamido)propanoate		Dabigatran Ethyl Ester, Process related and degradant impurity (oxidation)
Impurity-E Chemical Name: 3-(2-(((4-(N-((hexyloxy)carbonyl) carbamimidoyl)phenyl)amino) methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid		Dabigatran Etexilate acid. Degradant impurity (acid/Base) and Metabolite.
Impurity-H Chemical Name: (Z)-isopropyl-3-(2-(((4-(N'-((hexyloxy) carbonyl)carbamimidoyl)phenyl) amino)methyl)1-methyl-N-(pyridine-2-yl)-1H-benzo[d] imidazole-5-carboxamido)propionate		Process related Impurity.

(b)

Figure 1. (a) Chemical structure of DABE; (b) Chemical structure of DABE impurities.

2.2. Equipment

HPLC analysis was performed with a Waters (Milford, MA, USA) Alliance HPLC system equipped with Photodiode array detector. This system was controlled by Waters Empower software. An Inertsil ODS-3V column, 150 mm × 4.6 mm, 5 µm (GL sciences, California, USA) employed for chromatographic separation. All samples were centrifuged by Thermo Scientific multifuge machine. The specificity study was conducted by using heating oven, photo stability chamber and heating mantle (Thermo Lab, India).

2.3. Software/Data Treatment

Waters Empower 3 software (Milford, MA USA) was used to acquire, store, and process the chromatographic data. Stat-Ease Inc. Design-Expert 8 (Minneapolis, MN) was used for the DOE work.

2.4. Standard and Sample Preparation

Standard and Test samples were prepared in Water and Acetonitrile in ratio of 70:30 (v/v) as diluent. The im-

purity stock solution was prepared by dissolving an accurately weighed amount of impurity in diluent, resulting in a concentration of 100 µg·mL^{-1} of each impurity. The identification solution was prepared by dissolving 25 mg of DABE mixed with 1 mL of impurity stock solution and diluted to 50 mL in diluent. The working standard solution of DABE was prepared by dissolving an accurately weighed amount of DABE working standard in diluent, resulting in a concentration of 500µg·mL^{-1}. Then above solution was further diluted in diluent to get a final solution of 5 µg·mL^{-1}. The test solution was prepared by dissolving an accurately weighed portion of the capsule pellets, equivalent to 50 mg of DABE in 70 mL diluent. After sonicating for around 20 minutes, the volume was made up to 100 mL resulting in a concentration of 500 µg·mL^{-1}. The above solution was centrifuged at 5000 rpm for 5 minutes in order to eliminate insoluble excipients. The supernatant liquid was used for chromatographic analysis.

2.5. Chromatography

The analytes were separated on Inertsil ODS-3V, 150 mm × 4.6 mm, 5 µm column at column oven temperature of 35˚C with a gradient run program at a flow-rate of 1.2 mL·min^{-1}. 0.01 M Ammonium formate buffer, adjusted pH 4.7 with formic acid was used as mobile phase A and Acetonitrile was used as mobile phase B. The HPLC gradient program (T/%B) was set as 0.01/30, 10.0/55, 15.0/60, 15.1/30 and 20/30. The mobile phase was filtered through a 0.45 µm Millipore filter, before use. UV detection was performed at 220 nm. The sample injection volume was 10 µL.

2.6. Quality by Design

QbD is a systematic approach to development that begins with predefined objectives, that emphasizes product understanding and product controls based on sound science and quality risk management. QbD also assures to ensure to design a quality product that consistently delivers the intended performance.

Similar principles and concepts have been adopted for analytical method development, and termed Analytical QbD (AQbD). To obtain a robust method by defining an allowed design space, QbD is an intellectual approach for analytical methods. By this approach, method developers gain an in-depth understanding about the method by attaining expert domain knowledge. There are various ways to realize a QbD method. **Figure 2** parades one of the schematic diagrams by showing various steps in flow chart manner. QbD tools comparison between Product development and Analytical method development is illustrated in **Table 1**.

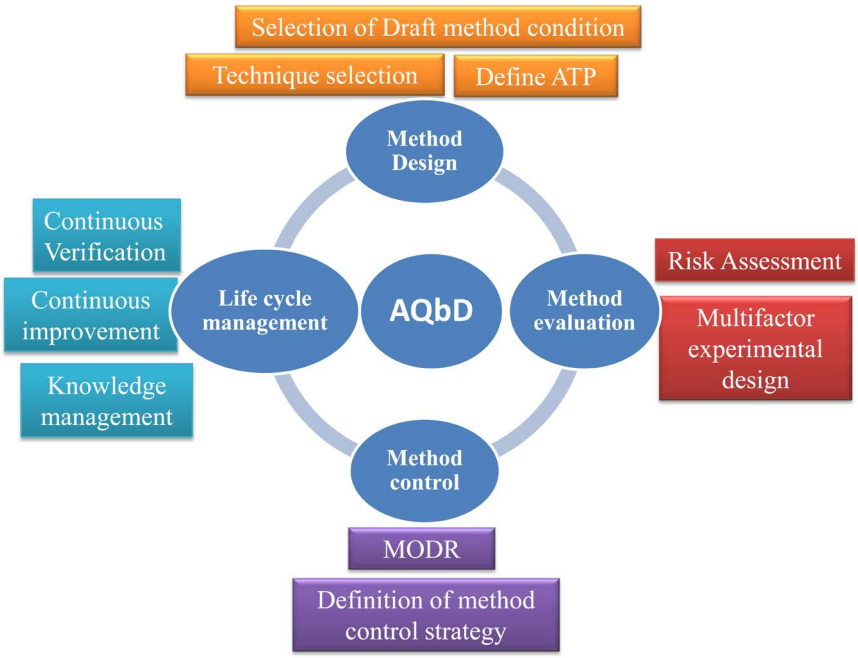


Figure 2. Analytical QbD flow chart.

Table 1. QbD tools comparison between Product development and analytical method development.

S. No.	Drug product	Analytical method	Assay	RS
1.	Quality target Product Profile (QTPP)	Analytical target profile (ATP)	• Accurate quantitation of Active substance • Shorter runtime • Stability indicating method. • Common method.	• Accurate quantitation of Impurities • Stability indicating method, • Resolution should be not less than 2.0
2.	Critical Quality Attributes (CQA)	Critical Method Attributes (CMA)	• Peak Tailing, • Recovery.	• Spectral peak purity, • Resolution between critical pair of peaks.
3.	Critical Process parameters (CMP)	Critical Method parameters (CMP)	• Flow, • Mobile phase pH • Column temperature • Diluent composition • Sonication time.	• Flow, • Mobile phase pH • Column temperature.
4.	Proven acceptable range (PAR)	Method operational design range (MODR)	Mobile phase pH ±0.2, Flow rate ±10%. Diluent composition range. Sonication time range.	Mobile phase pH ±0.2, Flow rate ±10%.

3. Method Design

3.1. Technique Selection

DABE and its impurities having chromophore group, so HPLC connected with UV detector analytical technique has been selected.

3.2. Analytical Target Profile

The ATP is a prospective summary of the requirements of a measurement system that, if achieved, will ensure an accurate assessment of a particular product quality attribute over the lifecycle of that product.

An example ATP is provided below for the assay and related substances of an immediate release capsules dosage form.

3.3. Assay

The procedure must be able to accurately quantify the API in capsules over the range 70% - 130% of the nominal concentration with specificity, linearity, accuracy, and precision such that measurements fall within ±3.0% of the true value with a 95% probability.

3.4. Related Substance

The procedure must be able to quantify specified and unspecified impurities relative to API in the presence of drug substance, excipients, and impurities over the range from reporting threshold to the specification limit. The specificity, linearity, accuracy, and precision of the method must be such that measurements fall within ±20% of the true value for impurity levels ≤0.15%, with 80% probability, and within ±15% of the true value for impurity levels >0.15%, with 90% probability.

3.5. Selection of Draft Method Conditions

DABE is a weak base (pKa1 = 4.0 ± 0.1 and pKa2 = 6.7 ± 0.1) with the partition coefficient of the free base of log P = 3.8. Based on literature, initial experiments were carried out using acidic pH buffer, L1 column, Acetonitrile (Low UV cutoff) with a gradient flow. To avoid DABE pKa, Initial experiments were started with pH 3.0 phosphate buffer as mobile phase-A and Acetonitrile as Mobile phase B, by using Hypersil and tagra columns, but Impurity C & B were co-eluting at acidic mobile phase pH. Phosphate buffer has three different pka values: 2.1, 7.2 and 12.3, so phosphate buffer pH changed to 6.3 to check the separation of impurity C&B. Significant peak separation observed between Impurity B & C at basic mobile phase pH. The methodology does not solely

focus on UV detection but also LCMS suitability to track the degradant components in forced degradation study.

Volatile buffer like ammonium acetate and ammonium formate shall be selected for mobile phase preparation. Mobile phase buffer changed from phosphate buffer to acetate buffer pH adjusted to 5.0, at this pH acetate buffer is active. Gradient program was optimized and performed method robustness parameters to check the method impacting factors. One major degradant which is forming during hydrolysis is eluting between DABE and impurity H. The unknown peak RT was changing as pH changes. At pH 5.2 DABE and major unknown are co-eluting; it indicates that method is very sensitive to pH of mobile phase. Mobile phase buffer is changed to formate buffer and pH adjusted to 4.5 to elute unknown impurity after Impurity H. In optimized condition all peaks are separated and unknown impurity is eluting very lately after impurity H within 20 mins gradient program. In this optimized method conditions the resolution between individual peak is more than 2.0, the peak purity of all peaks are passing *i.e.* purity angle is less than purity thresholdas per waters Empower software algorithm. The Method design chromatograms are presented in **Figure 3**.

4. Method Evaluation

4.1. Risk Assessment of Method Parameter

Risk assessment (RA) is a critical step in the QbD method development process. RA of the Method parameters (MP) was performed to evaluate the impact on CMAs. The relative risk of CMPs on CMAs was ranked as High, Medium and Low. The high risk parameters warranted further investigation whereas the low risk attributes required no further investigation. The medium risk is considered acceptable based on current knowledge. Further investigation for medium risk may be needed in order to reduce the risk. Based upon the initial method development trails, the risk assessment of method attributes is given in **Table 2**.

DABE is highly soluble in water and acetonitrile. Diluent (30% acetonitrile) is suitable to get sufficient recovery in capsules dosage form.

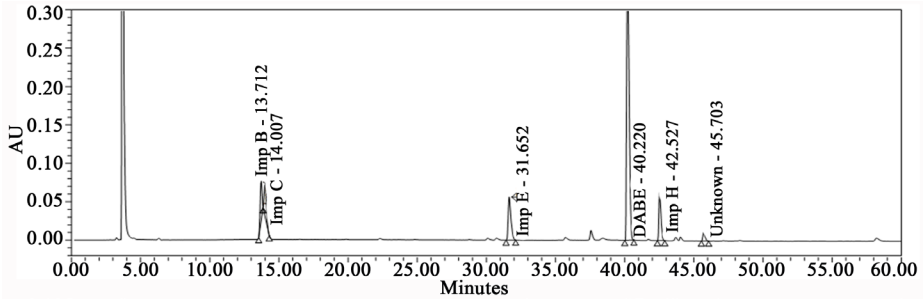
4.2. Summarized Quality Attributes (QA)

Critical method attributes (CMA) is an element of method performance that must be measured to assess whether a method is capable of producing fit-for-purpose data. Diversified responses ascertain the method's quality. However, the significantly varied response/s befit as CMA. Assuring to sustain the method's predefined ATPs, the responses for the peak of DABE and its impurities are:

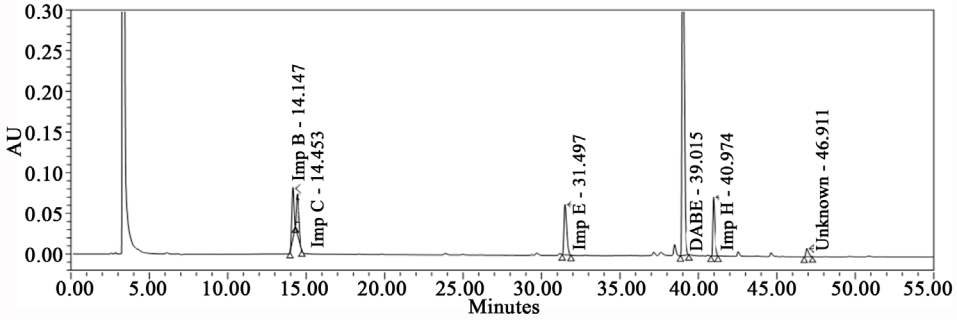
i. Resolution from closely eluting peak
ii. Theoretical plates (USP plate count)
iii. Tailing factor (USP tailing)

Table 2. Risk assessment of method attributes.

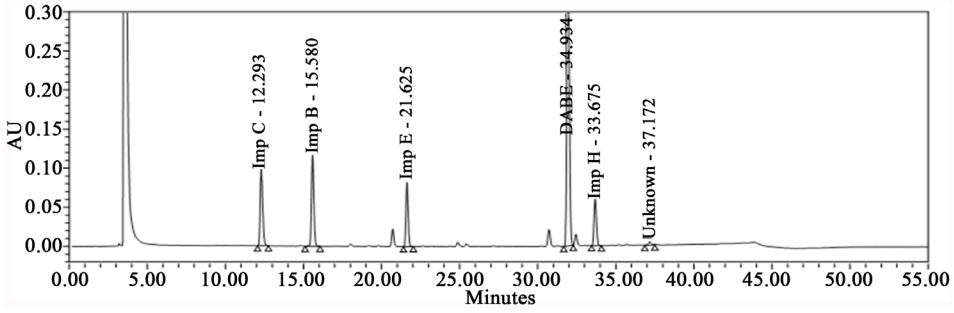
Critical method attributes	Critical method parameters					
	Flow	Column temperature	Mobile phase-A buffer concentration	Mobile phase-A pH	Mobile phase organic ratio	Injection volume
Resolution between Impurity C and B	High	High	Low	High	Low	Low
Resolution between Impurity DABE and H	Medium	Medium	Low	Medium	Low	Low
DABE RS Standard Tailing Factor	Medium	Medium	Low	Medium	Low	Low
DABE AY Standard Tailing Factor	Medium	Medium	Low	Medium	Low	Low
DABE AY Theoretical Plates	Medium	Medium	Low	Medium	Low	Low


AU
Minutes
Imp B - 13.712
Imp C - 14.007
Imp E - 31.652
DABE - 40.220
Imp H - 42.527
Unknown - 45.703

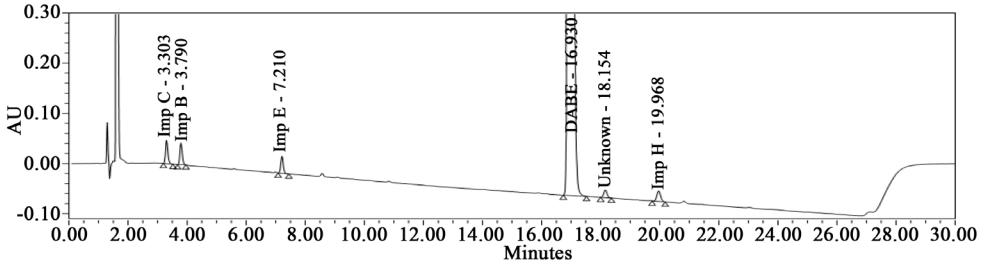
(a)


AU
Minutes
Imp B - 14.147
Imp C - 14.453
Imp E - 31.497
DABE - 39.015
Imp H - 40.974
Unknown - 46.911

(b)


AU
Minutes
Imp C - 12.293
Imp B - 15.580
Imp E - 21.625
DABE - 34.934
Imp H - 33.675
Unknown - 37.172

(c)


AU
Minutes
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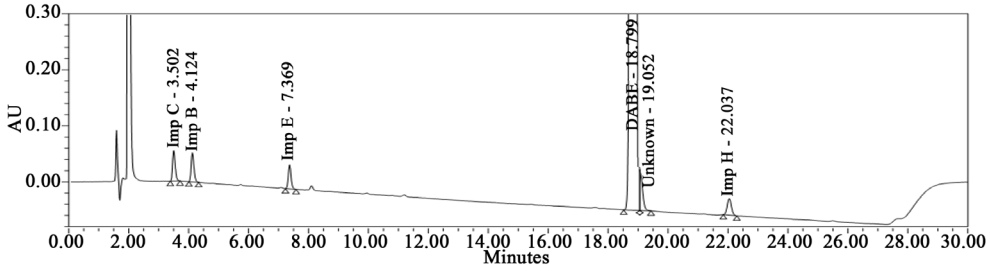
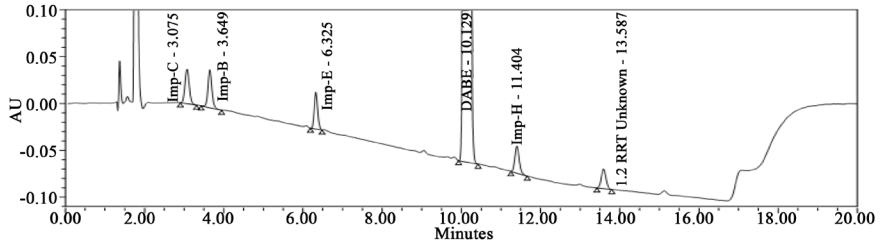
Imp B - 3.790
Imp E - 7.210
DABE - 16.930
Unknown - 18.154
Imp H - 19.968

(d)


AU
Minutes
Imp C - 3.502
Imp B - 4.124
Imp E - 7.369
DABE - 18.799
Unknown - 19.052
Imp H - 22.037

(e)



(f)

Figure 3. Method optimization: (a) pH 3.0 phosphate bufferHypersil column; (b) pH 3.0 phosphate buffer Tagra column, (c) pH 6.3 phosphate buffer Xterra column. Method optimization: (d) pH 5.0 ammonium acetate buffer; (e) pH 5.2 ammonium acetate buffer; (f) impurity spiked chromatogram at center point method conditions (pH4.5 formate buffer, Intertsil ODS, 150 × 4.6, 5 µm).

4.3. Multifactor Experimental Design

4.3.1. Center Point Selection

Based on method design, following method conditions are selected as center point. Mobile phase A consists of a pH4.5. 10 mM Ammonium formate buffer and mobile phase B consists of acetonitrile. Flow rate 1.0 mL/min with Inertsil ODS3V 150 mm × 4.6 mm, 5 µm column, 30˚C column temperature. The HPLC gradient program (T/%B) was set as 0.01/30, 10.0/55, 15.0/60, 15.1/30 and 20/30.

4.3.2. Factor and Response Selection

Selected the Critical Method parameters (CMP) and Method attributes (responses) identified from the RA and set low and high factor limits for experimentation along with acceptance limits for attributes. Five responses are evaluated; R1, R2, R3, R4 and R5. R1 is Resolution between Imp C and Imp B. R2 is resolution between DABE and Imp-H. R3 is Tailing factor of DABE RS standard. R4 is Tailing factor of DABE Assay standard. R5 is theoretical plates of DABE Assay standard. R1, R2 and R3 represent the RS method performance design. R4 & R5 represents Assay method performance design. In single DOE, both Assay and RS design space will be established. The parameters (factors) and attributes (responses) are presented in **Table 3** & **Table 4** along with experimental ranges investigated. Buffer concentration are set to fixed values and hence not evaluated experimentally within the design.

4.3.3. DOE Design Selection and Design Layout

Selected and generated a statistical design for the factors that allows the determination of important main effects and two-factor interactions using an appropriate statistical software package Design-Expert. The number of factors being 3, a full factorial design was proposed. A full factorial design apart from being a simple design would also facilitate in obtaining maximum information regarding factors, and factor interaction effects on the responses in as less as 8 experimental runs. Further factor study could also be done by augmenting the design to a higher order design. Three center points runs added were evenly distributed through the entire experimental run sequence to provide a means to test for a systematic time effect that may have occurred during the experiment. The absence of such an effect allows inferences to be drawn concerning the effect of temperature with no concern of confounding with the experimental run sequence

4.3.4. DOE HPLC Analysis

Instrument methods were generated in Empower to support factor variations for each of the design points. Samples were evaluated under each design point and response results gathered, and summarized for statistical analysis in **Table 5**.

4.3.5. DOE Statistical Response Analysis

Each of the responses was analyzed using Design Expert 8 software. The p values from the corresponding ANOVA (adjusted for curvature) for each response are presented in **Table 6**. The level of significance was determined based on the p values. A response term with p value less than 0.05 was considered significant and for a response term with p value more than 0.05 was considered not significant. Model for response R3 was observed

Table 3. Factors and their levels of study.

S. No.	Factors	Units	Levels of study		
			−1	0	+1
1	Flow	mL/min	0.8	1.0	1.2
2	Temp	˚C	25	30	35
3	pH	-	4.3	4.5	4.7

Table 4. Acceptance criteria for the responses.

Code	Responses	Acceptance criteria
R1	Rs Imp C and Imp B	Not less than 2.0
R2	Rs DABE and Imp H	Not less than 2.0
R3	RS DABE TF	Not more than 1.5
R4	Assay DABE TF	Not more than 1.5
R5	Assay DABE TP	Not less than 5000

Table 5. Design of the experiments and observed responses.

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
		A:Flow	B: Temperature	C: Mobile phase A pH	Rs Imp C & Imp B	Rs DABE & Imp H	RS DABE TF	AY DABE TF	AY DABE TP
7	1	0.8	35	4.7	4.72	6.22	1.09	1.02	56,339
6	2	1.2	25	4.7	3.43	6.19	1.09	1.06	42,754
8	3	1.2	35	4.7	4.67	6.53	1.07	1.02	49,162
1	4	0.8	25	4.3	1.74	5.45	1.14	1.15	45,773
5	5	0.8	25	4.7	3.49	5.63	1.12	1.06	49,076
3	6	0.8	35	4.3	2.7	6.01	1.13	1.11	52,866
9	7	1	30	4.5	2.97	6.07	1.12	1.08	46,174
11	8	1	30	4.5	2.98	6.14	1.11	1.08	46,515
10	9	1	30	4.5	3.06	6.17	1.1	1.08	46,662
2	10	1.2	25	4.3	1.74	5.94	1.11	1.14	40,369
4	11	1.2	35	4.3	2.52	6.45	1.08	1.11	46,234

Table 6. *p*-value for responses.

	R1: *p*-value	R2: *p*-value	R3: *p*-value	R4: *p*-value	R5: *p*-value
Source	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Model	0.0018	0.0184	0.1445	<0.0001	0.0023
A: Flow	0.1732	0.0064	0.0442	<0.0001	0.0008
B: Temperature	0.0011	0.0052	0.0862	<0.0001	0.0007
C: Mobile phase A pH	0.0003	0.0383	0.0862	<0.0001	0.0034
AB	0.3473	0.1747	0.7575	<0.0001	0.0987
AC	0.6657	0.7194	0.4	<0.0001	0.1748
BC	0.0346	0.4365	0.7575	<0.0001	0.42
ABC	0.3064	0.3021	0.4	<0.0001	0.6509
Curvature	0.0665	0.1663	0.4534	<0.0001	0.0149

to be not significant; holding a p value of 0.1445 that is greater than 0.05, which implies that response measured did not had significant impact by the studied range of factors.

Curvature was observed to be significant for response R4 and R5 (<0.05), indicates that the design considered did not sufficiently explain the effect of the factors on the respective responses. Curvature being significant indicates that a higher order model would be necessary to accurately represent the response.

4.3.6. DOE Augmentation

The design studied was 2 level full factorial design with 3 factors (2^3). This implies 8 runs (not counting the 3 center points). Graphically it can be represented in **Figure 4**. The pictorial representation shows that the runs performed occupied all the corners of the cube, where all the three factors change simultaneously. Face centered central composite design (FC CCD) was proposed for better understanding on effect of factors on the responses. In FC CCD a run was added to the center of each face, where one of the 3 factors is fixed at the center point value resulting in addition of 6 experiments. The pictorial representation of augmented design is in **Figure 5**. The complete augmented design with responses is presented in **Table 7**. Each of the responses was analyzed using Design Expert 8 software. p values from ANOVA for each response were presented in **Table 8**. The p value of Model for each response is less than 0.05, implying Model is significant. Factor terms and 2 factor interaction terms with p value less than 0.05 are significant. 3 factor interactions were not significant for any of the responses obtained. Lack of fit was also observed to be not significant for all the response. This further assures the suitability of model for optimization use. Contour plot for responses R1, R2, R3, R4 & R5 represented in **Figures 6-10** respectively.

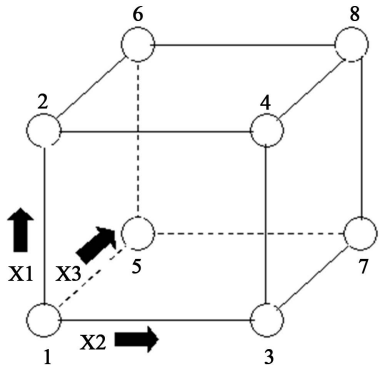


Figure 4. 2 factors full factorial design.

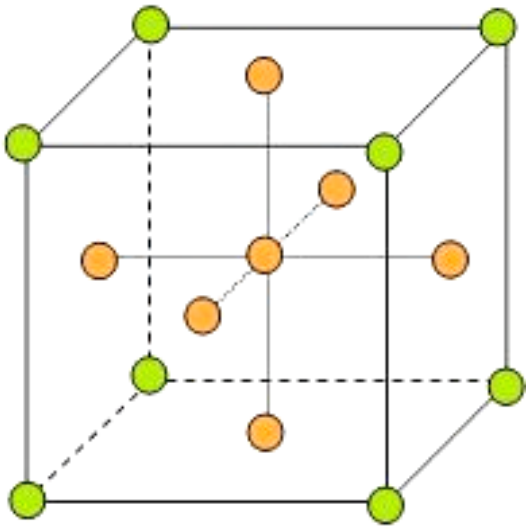


Figure 5. 2 factors full factorial design augmented to Face centered central composite design.

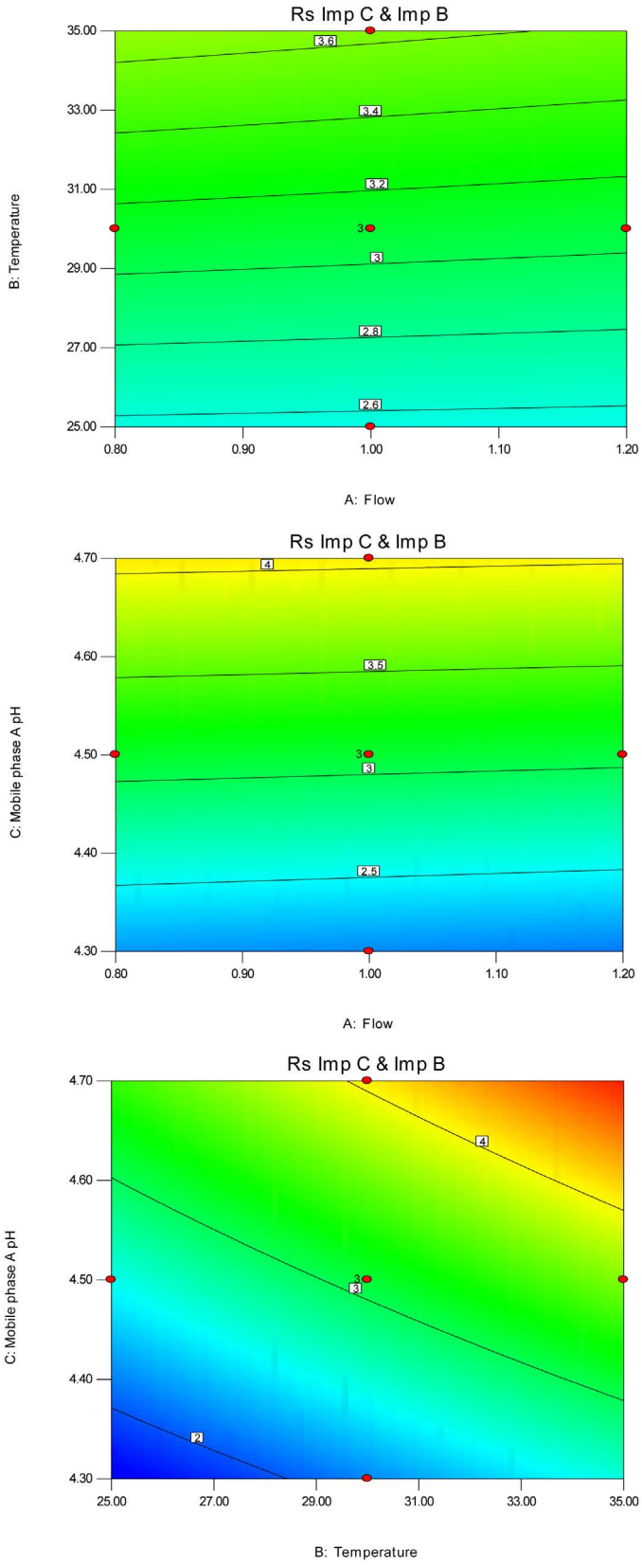


Figure 6. Contour plots for R1-Rs Imp C & Imp B.

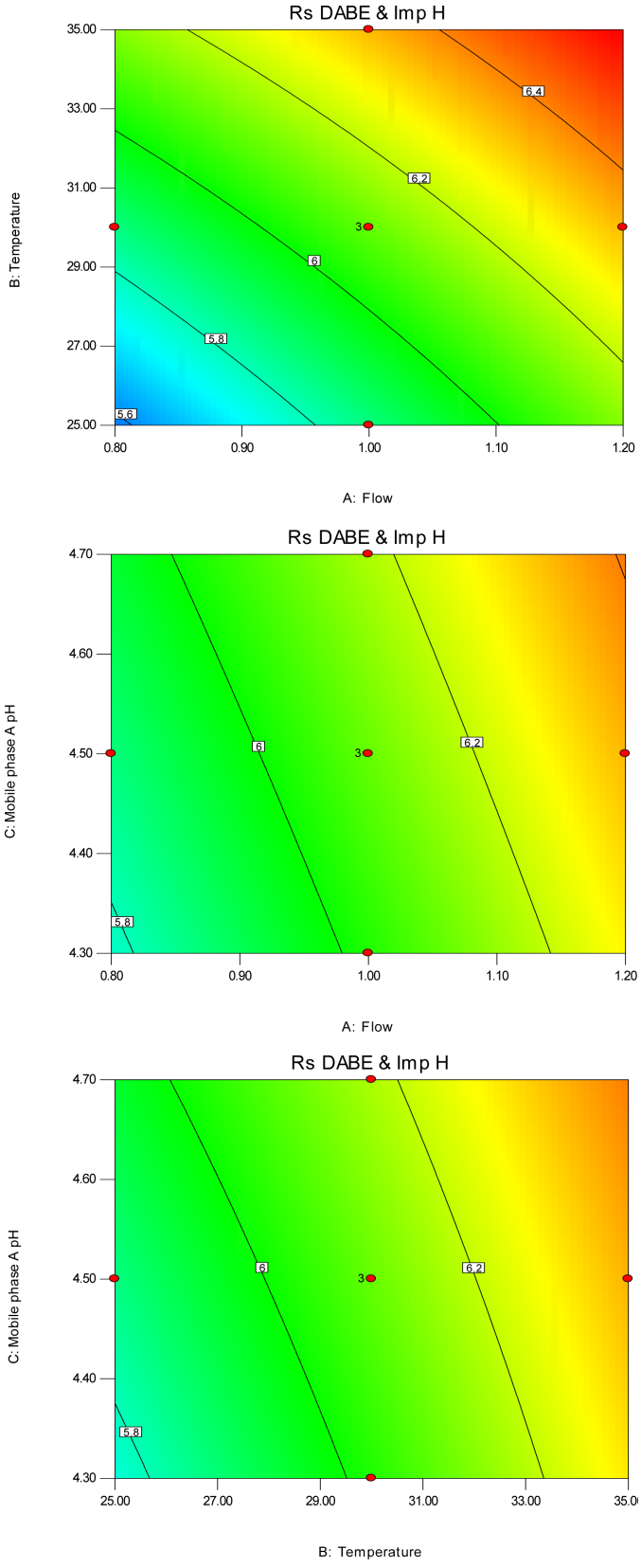


Figure 7. Contour plots for R2-Rs DABE & Imp H.

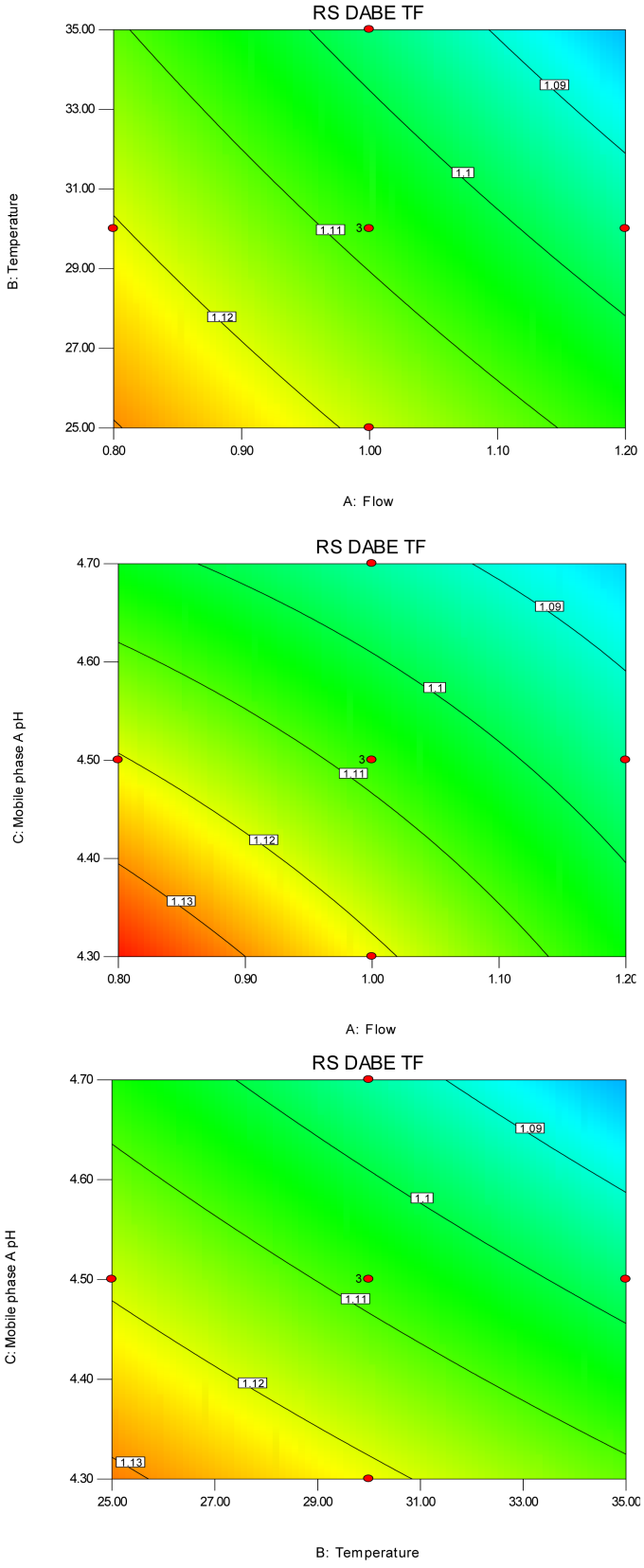


Figure 8. Contour plots for R3-RS DABE TF.

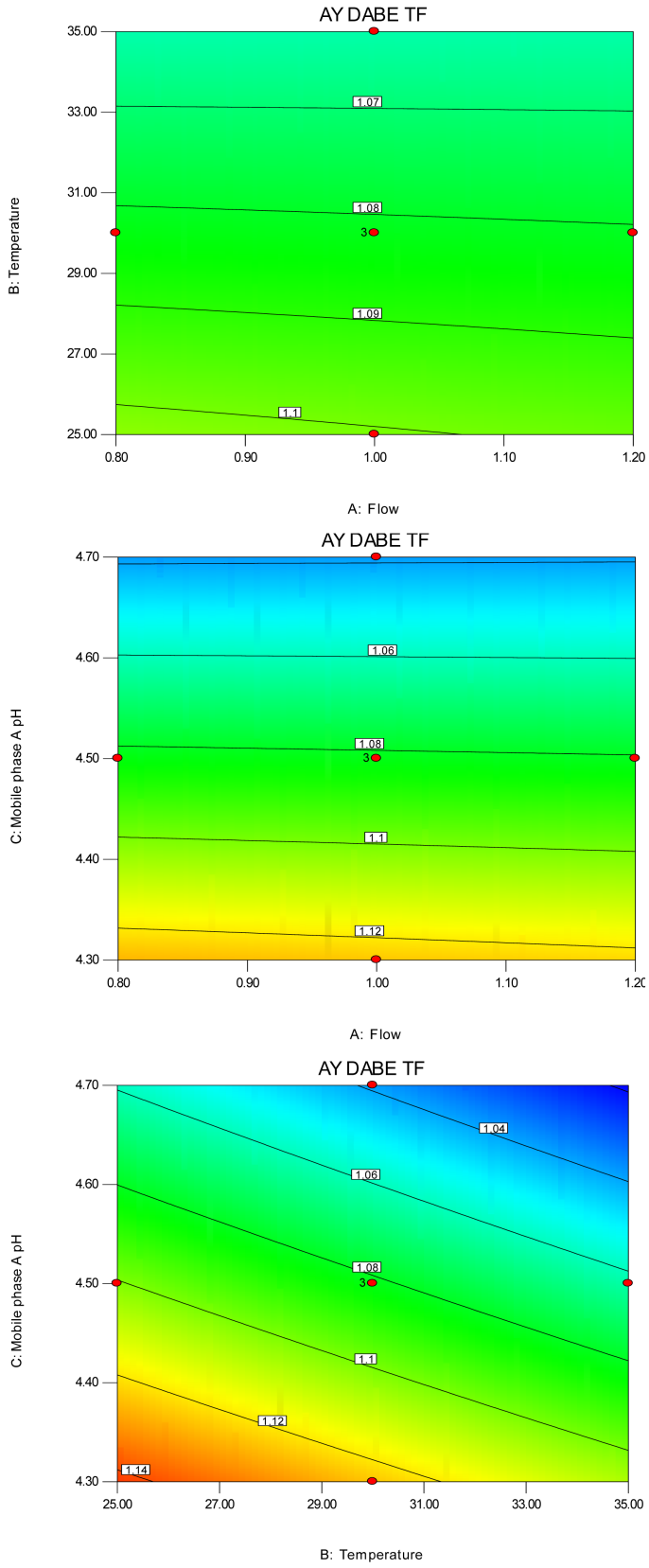


Figure 9. Contour plots for R4-AY DABE TF.

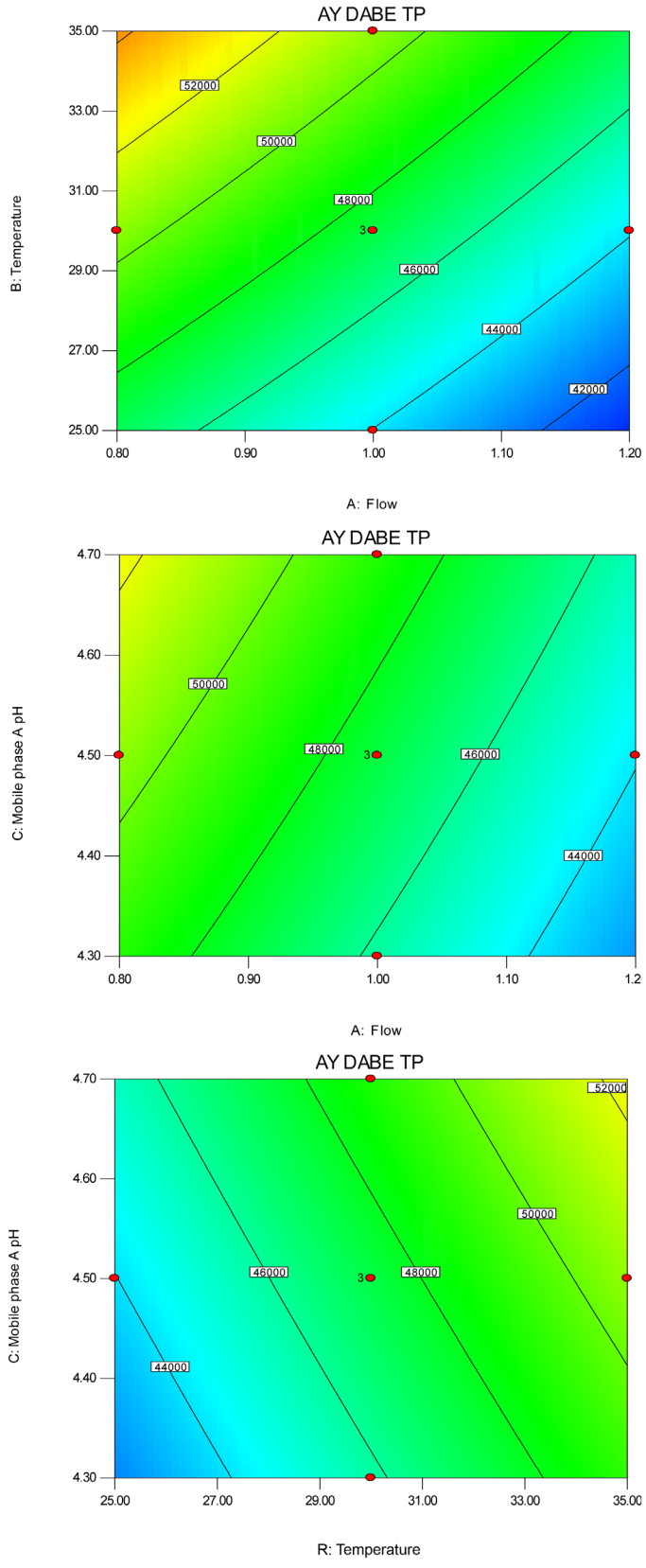


Figure 10. Contour plots for R5-AY DABE TP.

Table 7. Complete augmented design with responses.

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
		A:Flow	B: Temperature	C:Mobile phase A pH	Rs Imp C & Imp B	Rs DABE & Imp H	RS DABE TF	AY DABE TF	AY DABE TP
7	1	0.8	35	4.7	4.72	6.22	1.09	1.02	56,339
6	2	1.2	25	4.7	3.43	6.19	1.09	1.06	42,754
8	3	1.2	35	4.7	4.67	6.53	1.07	1.02	49,162
1	4	0.8	25	4.3	1.74	5.45	1.14	1.15	45,773
5	5	0.8	25	4.7	3.49	5.63	1.12	1.06	49,076
3	6	0.8	35	4.3	2.7	6.01	1.13	1.11	52,866
9	7	1	30	4.5	2.97	6.07	1.12	1.08	46,174
11	8	1	30	4.5	2.98	6.14	1.11	1.08	46,515
10	9	1	30	4.5	3.06	6.17	1.1	1.08	46,662
2	10	1.2	25	4.3	1.74	5.94	1.11	1.14	40,369
4	11	1.2	35	4.3	2.52	6.45	1.08	1.11	46,234
12	12	0.8	30	4.5	3.09	5.84	1.11	1.08	50,272
13	13	1.2	30	4.5	3.05	6.43	1.11	1.08	43,373
14	14	1	25	4.5	2.48	5.96	1.12	1.1	43,178
15	15	1	35	4.5	3.66	6.39	1.1	1.06	50,296
16	16	1	30	4.3	2.19	6.13	1.14	1.12	46,166
17	17	1	30	4.7	4.13	6.17	1.09	1.04	49,514

Table 8. *p*-value from ANOVA for each response.

Source	R1: *p*-value	R2: *p*-value	R3: *p*-value	R4: *p*-value	R5: *p*-value
	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Model	<0.0001	<0.0001	0.0107	<0.0001	<0.0001
A: Flow	0.2197	<0.0001	0.0061	0.3358	<0.0001
B: Temp	<0.0001	<0.0001	0.0145	<0.0001	<0.0001
C-pH	<0.0001	0.0176	0.004	<0.0001	0.0003
AB	0.4668	0.2324	0.766	0.285	0.4035
AC	0.7615	0.8037	0.3815	0.285	0.5533
BC	0.0098	0.5649	0.766	0.285	0.7709
ABC	0.4178	0.4155	0.3815	0.285	0.8787
Lack of Fit	0.2712	0.268	0.473	-	0.0674

R1 contour plots suggest, Resolution between Imp C and Imp B increase with increase in column temperature and pH of mobile phase A, whereas, flow rate has minimal or no effect within the studied range.R2 contour plots suggest resolution between DABE and Imp H increase with increase in column temperature, pH of mobile phase A and flow rate. The contour plot also suggests that, within the studied ranges of the factors the response values obtained are well above the acceptance criteria. R3 contour plots suggest RS DABE standard Tailing factor de-

crease with increase in column temperature, pH of mobile phase A and flow rate. The contour plot also suggests that, within the studied ranges of the factors the response values obtained are well below the acceptance criteria. R4 contour plots suggest, Assay DABE standard Tailing factor decrease with increase in column temperature and pH of mobile phase A, whereas flow rate has minimal effect within the studied range. R5 contour plots suggest Assay DABE standard Theoretical Plates increase with increase in column temperature and pH of mobile phase A whereas increase in flow rate decreases Assay DABE TP. Further it was also observed that, the response values obtained for the studied range of the factor were well above the acceptance criteria.

4.3.7. DOE Optimization

The method makes use of an objective function, called the desirability function. It reflects the desirable ranges for each response. The desirable ranges are from zero to one (least to most desirable, respectively).From the above observations it was evident, that among the three factors studied; most significant factors were the column temperature and pH of mobile phase A, whereas flow rate appeared to be of lesser significance. Hence during optimization study, flow rate was fixed at 1.0 mL/min, the acceptance criteria for all the response were set in the software. The Contour plot for Desirability represented in **Figure 11**. It shows increase in column temperature and the pH of mobile phase A, increases desirability, which indicates improvement in the acceptability of the responses measured. Even though flow rate is not having significant impact on responses, flow rate was increased to 1.2 mL/min to ensure elution of lately eluting degradation impurities which may form in DABE capsules formal stability study. Modified the Contour plot by fixing the Flow rate at 1.2 mL/min, and evaluate for desirability. The modified Contour plot for evaluation of Desirability presented in **Figure 12**. The above contour plot shows increase in column temperature and the pH of mobile phase A at flow rate of 1.2 mL/min, increases desirability, which indicates improvement in the acceptability of the responses measured. The other plots like Perturbation plot, 3D plot and design space was represented in **Figure 13**.

5. Method Control

5.1. MODR Determination

MODR is defined by the actual linear equations for the critical responses set to their respective acceptance limit, with respect to the method factor input values. Therefore, not all combinations of factor values set by the opera-

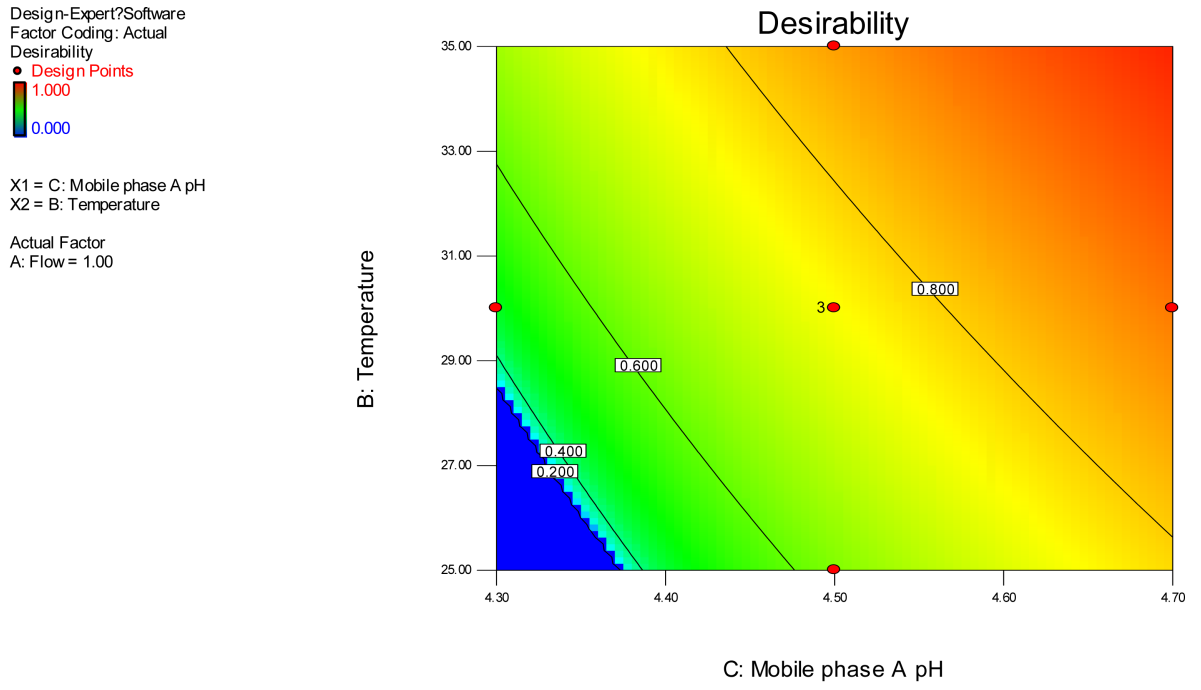


Figure 11. Contour plot for Desirability at flow rate 1.0 mL/min.

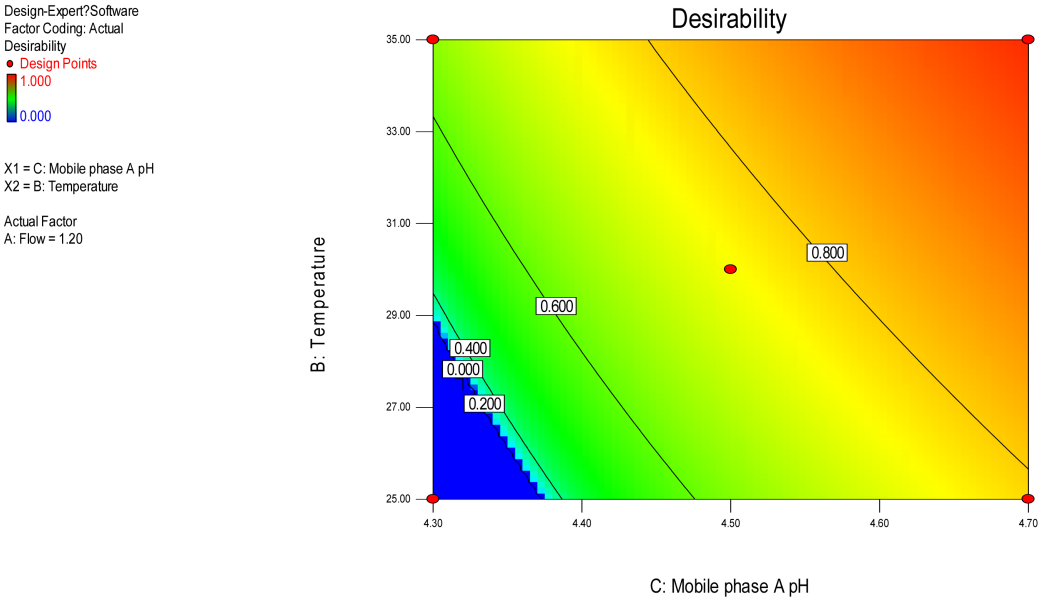
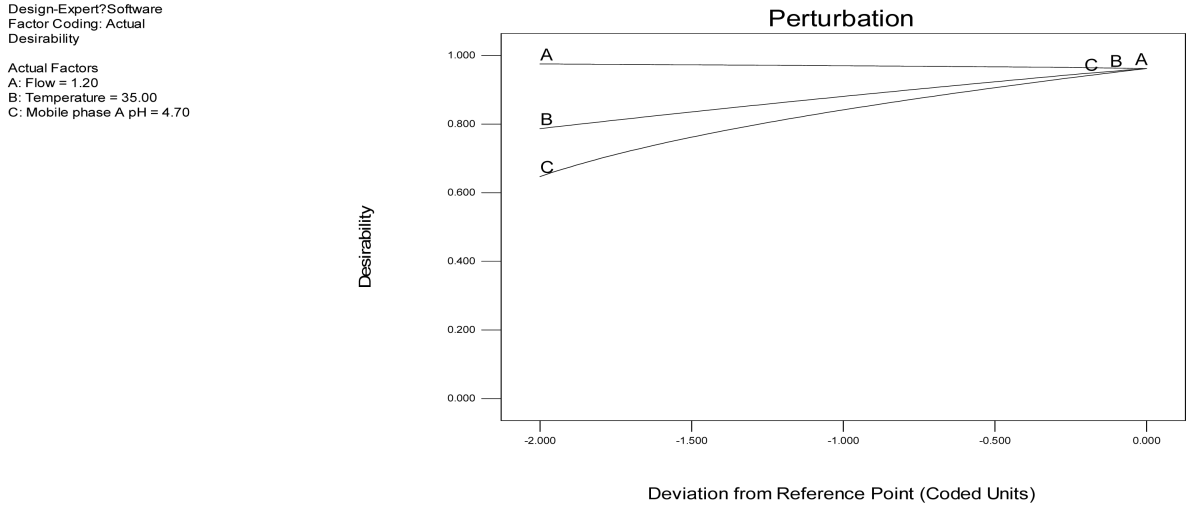
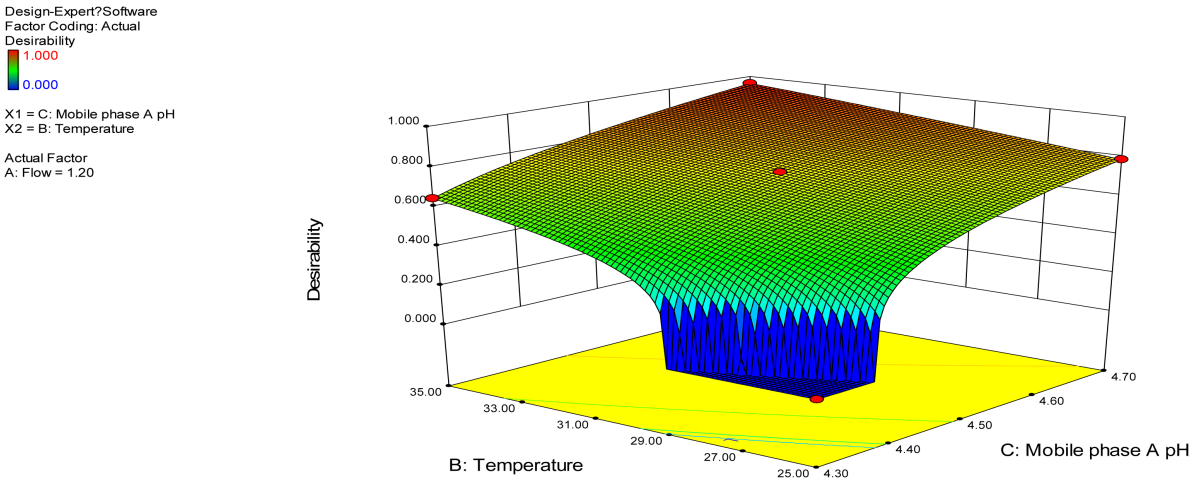


Figure 12. The modified contour plot for evaluation of desirability at flow rate 1.2 mL/min.





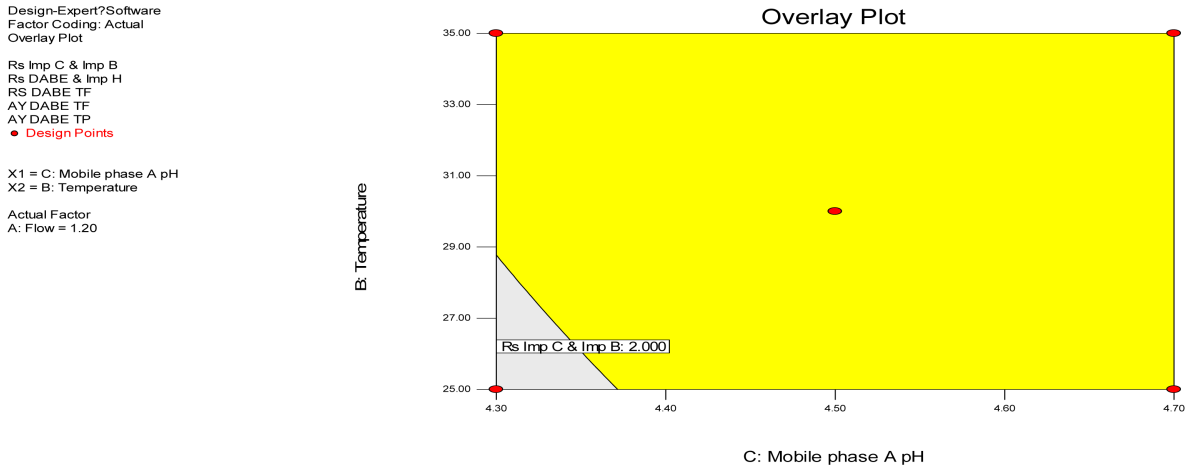


Figure 13. (a) Perturbation plot; (b) 3 D plot c. design space.

ble ranges presented in **Table 9** may result in tenable solutions. As iterated above the multidimensional nature of the study doesn't readily or easily lend itself to a two-dimensional presentation. A chromatogram of the final set point conditions is shown in **Figure 14**. As illustrated in **Table 10**, there is excellent agreement between the response model predictions and the actual results obtained for the final method conditions, indicating that the model is capable of navigating and accurately predicting the multivariate design space.

5.2. Method Validation

In current scenario the optimized parameters form the extreme of studies range of variables which further suggest for method validation to ensure method intent. The method was validated for specificity, precision, accuracy, sensitivity and linear range as per the International Conference on Harmonization (ICH) guidelines.

5.3. System Suitability

Injected diluent as blank, identification solution, three standard solutions and calculate% RSD.

5.4. Specificity

Separate portions of drug product and ingredients were exposed to following stress conditions to induce degradation.

The placebo (excipients without active) solution was prepared by dissolving an accurately weighed portion of the powder equivalent to 50 mg of DABE in 80 mL diluent. After sonicating for around 20 minutes, the volume was made up to 100 mL.

The drug product was subjected to base hydrolysis using 1 N Sodium hydroxide at 60˚C for duration of 15 minutes, acid hydrolysis with 1 N Hydrochloric acid at 60˚C for duration of 2 hours and neutral hydrolysis with water at 75˚C for duration of 3 hours. Oxidation study was performed with 3% Hydrogen Peroxide solution at 75˚C for 2 hours. On photo stability study, drug product was sufficiently spread on Petri plates (1 mm thick layer), exposed to sunlight (1.2 million lux hours) and UV light (200 wats/m^2). Humidity study was performed separately by exposing the drug product to humidity at 25˚C, 90% RH for 7 days. Thermal degradation study was performed by heating drug product at 70˚C for 48 hours. Similarly placebo samples were prepared as like as drug product by exposing formulation matrices without drug substance.

Physically stressed (photolytic, heat and humidity stressed sample and placebo prepared as per method conditions, chemically stressed sample prepared by dissolving an accurately weighed portion of the capsules pellets , equivalent to 50 mg of DABE in 80 mL diluents, after sonicating for around 20 minutes, add 5 mL reagent (1 N Hydrochloric acid, 1 N Sodium hydroxide, water and 3% peroxide) kept for above conditions kept at room temperature to attain room temperature neutralized it (for acid or base sample) the volume was made up to 100 mL resulting in a concentration of 500 $\mu g \cdot mL^{-1}$. Stressed samples were injected into the HPLC system with photo diode array detector and evaluated as per method conditions.

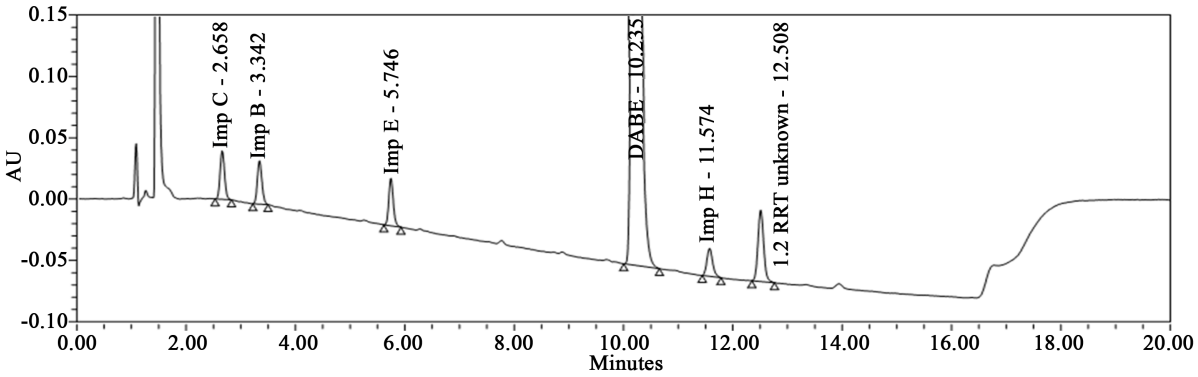


Figure 14. Impurity Spiked sample on water stressed sample in optimized method.

Table 9. Experimental DOE chromatographic conditions and MODR.

Method parameters (factors)	Units	(Old) Target	(Revised) Target	Operational range	
				LOW	High
Flow	mL/min	1.0	1.2	0.8	1.2
Temperature	˚C	30	35	25	35
Mobile phase A pH	N/A	4.5	4.7	4.4	4.7

Table 10. Experimental DoE MODR response model verification.

S. No.	Response	Centre points (avg.)	Predicted center points	Response model prediction			Proposed point actual	% Difference predicted vs. actual
				Proposed point prediction	95% CI low	95% CI high		
1	Rs Imp C and Imp B	3.00	3.09	4.66	4.49	4.82	4.57	2
2	Rs DABE and Imp H	6.13	6.10	6.57	6.40	6.75	6.78	3
3	RS DABE TF	1.11	1.11	1.07	1.05	1.10	1.10	3
4	AY DABE TF	1.08	1.08	1.02	1.01	1.03	1.10	8
5	AY DABE TP	46,450	47,336	48,704	46,943	50,465	50,206	3

5.5. Precision

The precision of test method was evaluated by using six samples of DABE tablet test preparation, spiking 0.5% of target concentration (500 µg·mL^{-1}) with impurities blend solution to get the concentration of 2.5 µg·mL^{-1} of each impurity and analyzed as per test method.% RSD of area% for each impurity was calculated. Intermediate precision was also studied using different column and performing analysis on different day. Similarly Six replicate sample solutions were prepared for the estimation of assay, the percent assay for each replicate, the average for 6 replicates and its% RSD were calculated.

5.6. Accuracy

To confirm the accuracy of the proposed method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking impurities in test preparation at the level of Limit of Quantification, 50%, 100%, 150% and 200% (A nominal concentration of about 1.25 µg·mL^{-1} to 5 µg·mL^{-1}) of the standard concentration. The accuracy of the assay was evaluated in triplicate at five different concentration levels (50% to 150%) by using DABE capsules pellets

5.7. Sensitivity

Sensitivity of the method was established with respect to Limit of detection and limit of quantification for DABE peak and its impurities. Series of concentration of drug solution and its impurities were injected; limit of detection (LOD) and limit of quantification (LOQ) was established by visual method. LOQ were experimentally verified by injecting six replicate injection of each impurity at the concentration obtained from above formula.

5.8. Linearity of Detector Response

A series of solutions of DABEimpurities in the concentration ranging from limit of quantification level to 200% (5.0 $\mu g \cdot mL^{-1}$) of standard concentration were prepared and injected into the HPLC system. Linearity test solutions for the assay method were prepared from the DABE stock solution at six concentration levels from 50% to 150% of the assay analyte concentration.

5.9. Robustness

To determine the robustness of the method, experimental conditions were deliberately changed and the tailing factors and resolution, RRT for DABE and its impurities were recorded. The flow rate of the mobile phase was changed by varying 0.2 units and verified at 1.0 and 1.4 mL/min. The effect of the column temperature on the resolution was studied at 30˚C and 40˚C instead of at 35˚C. The effect of pH of mobile phase buffer was studied by varying pH ± 0.1 units of method pH (4.7) keeping other mobile phase components constant.

5.10. Solution Stability

The solution stability of DABE in the assay method and DABE and its impurities in the related substance method were carried out by leaving both of the test solutions of the sample and standard solutions in tightly capped volumetric flasks at room temperature for 24 h. The same sample solutions were tested for every 6 h Interval up to 24 h.

5.11. Definition of Method Control Strategy

The next step is to evaluate method control strategy to determine if the method serves its intended goals. System suitability should be implemented to ensure that the desired method attributes are delivered.

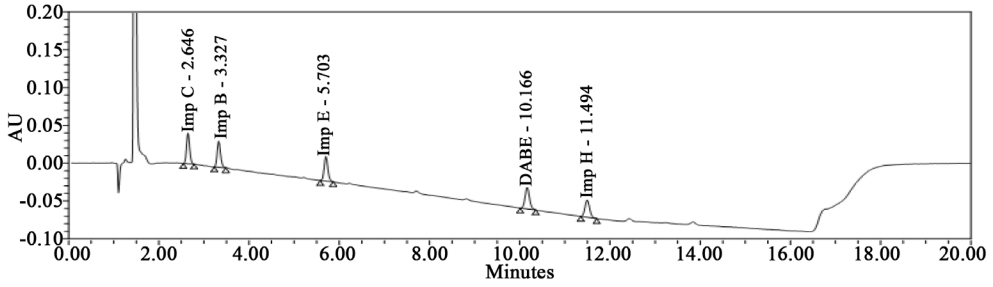
5.12. Life Cycle Management

One of the key components of QbD-based analytical method development is continual improvement. Post method verification, the analytical method is used for analysis and batch release, leading to generation of historical data. Thus the generated historical data is further evaluated for its performance and trend as a part of knowledge management. A need for method improvement is triggered based on the method performance being monitored and the trend analysis results. Additionally method improvement could also be triggered based on events, such as updated regulatory expectations, changes in the process, changes in the formula components, etc. as a part of life cycle management.

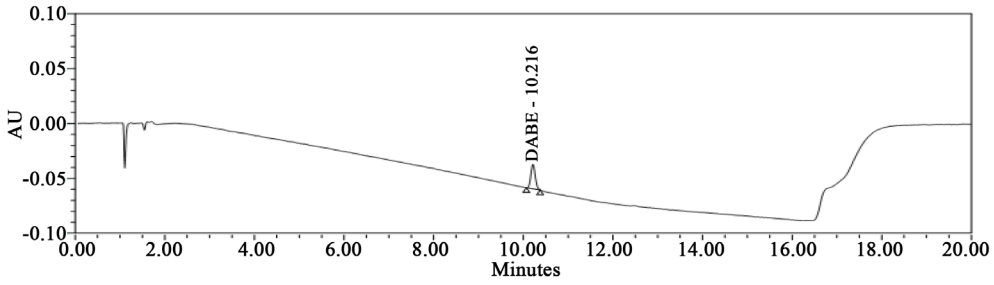
6. Results and Discussion

DOE optimized method has been proved to be a promising tool for separation of DABE and its impurities. DABE its impurities were well separated with good peak shape and resolution. No interfering peaks were observed in blank & placebo, indicating that signal suppression or enhancement by the product matrices was negligible.

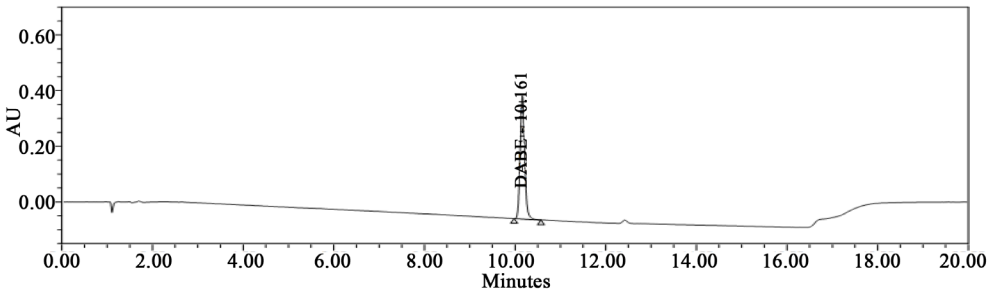
After satisfactory method optimization it was subjected to method validation as per ICH guideline. The method was validated to demonstrate that it is suitable for its intended purpose. The result of system suitability parameter was found to be complying with acceptance criteria: relative standard deviation standard area of replicate injection is not more than 5.0% and resolution between Imp C and Imp B is more than 2.0 and relative retention time of impurity peak should comparable as shown in **Table 11**. Impurity spiked standard, RS standard and Assay standard presented in **Figure 15**. The result of specificity study ascertained the separation of degradation peaks from DABE peak and the spectral purity of all exposed samples were found spectrally pure and data of degradation studies are shown in **Table 12**. Forced degradation chromatograms are presented in **Figure 16**.



(a)

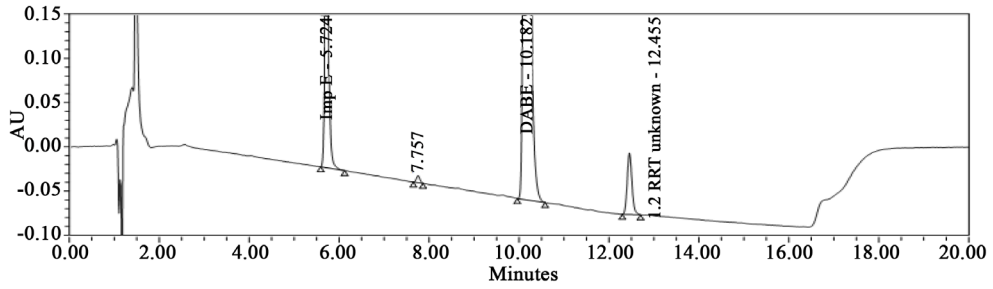


(b)

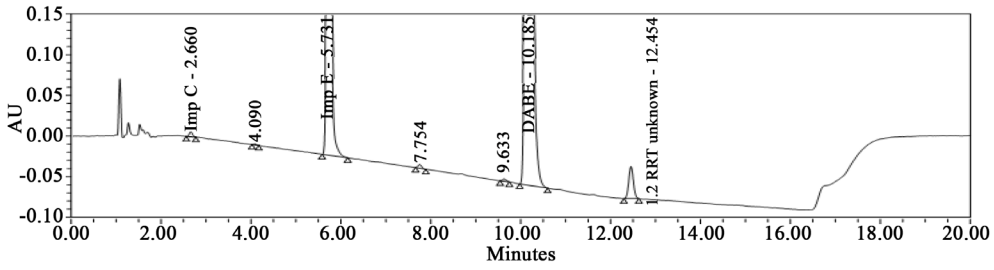


(c)

Figure 15. (a) DABE and its impurities; (b) RS standard; (c) Assay standard.



(a)



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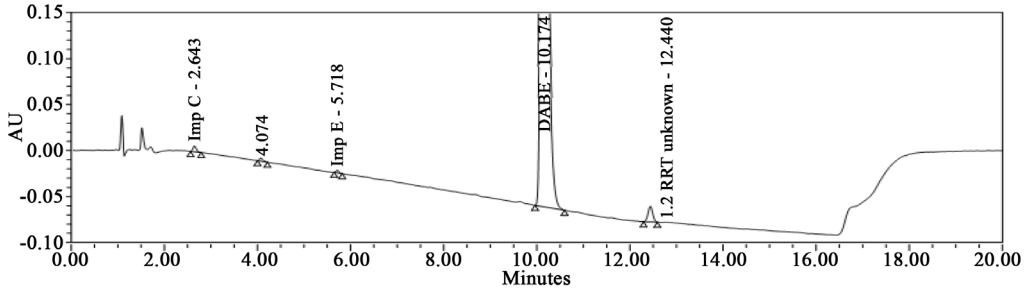
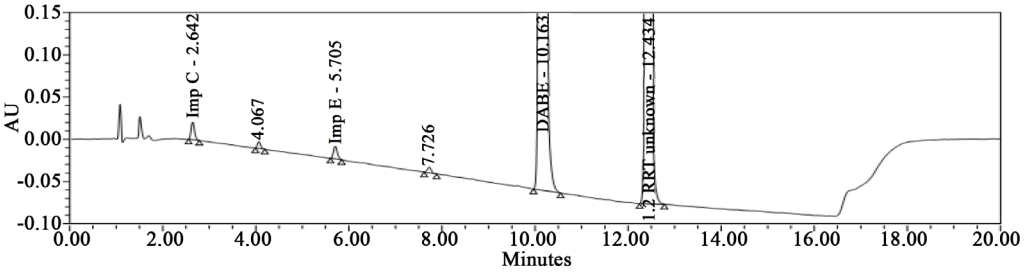

AU
Minutes
Imp C - 2.643
4.074
Imp E - 5.718
DABE - 10.174
1.2 RRT unknown - 12.440

(c)


AU
Minutes
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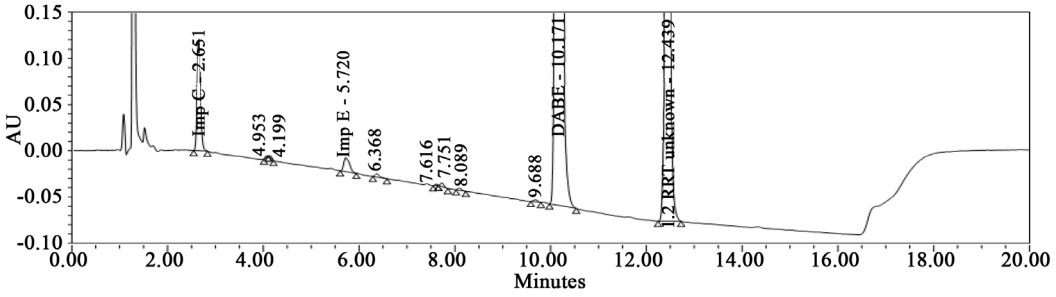
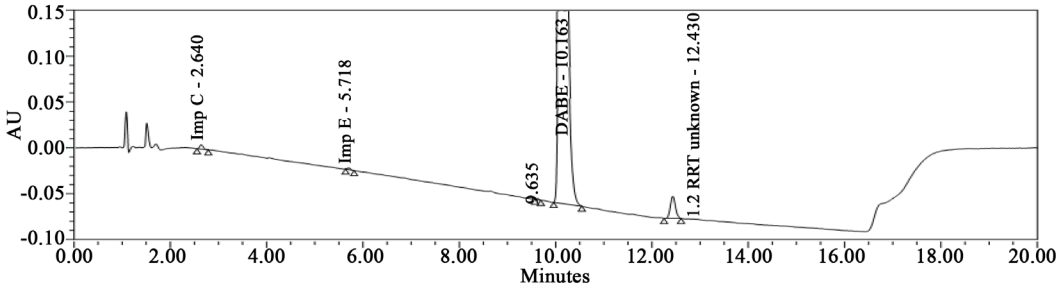
Imp E - 5.705
7.726
DABE - 10.163
1.2 RRT unknown - 12.434

(d)


AU
Minutes
Imp C - 2.651
4.953
4.199
Imp E - 5.720
6.368
7.616
7.751
8.089
9.688
DABE - 10.171
1.2 RRT unknown - 12.439

(e)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Minutes
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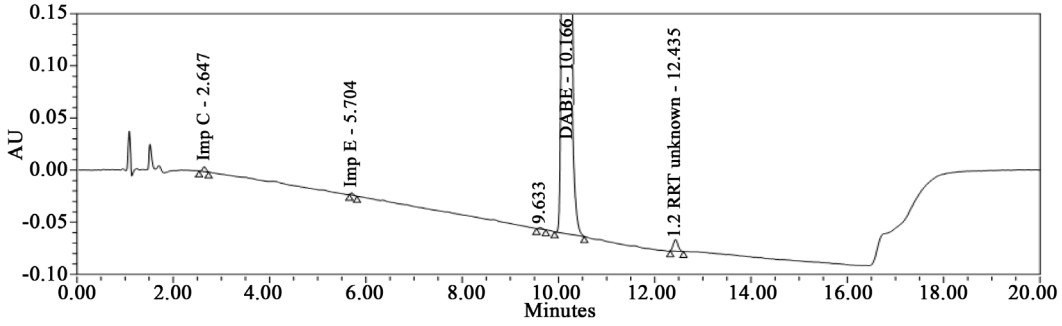
Imp E - 5.718
9.635
DABE - 10.163
1.2 RRT unknown - 12.430

(f)


AU
Minutes
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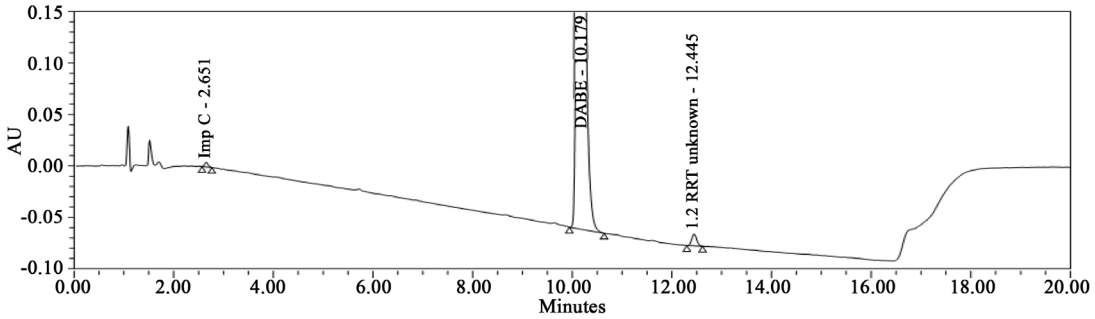
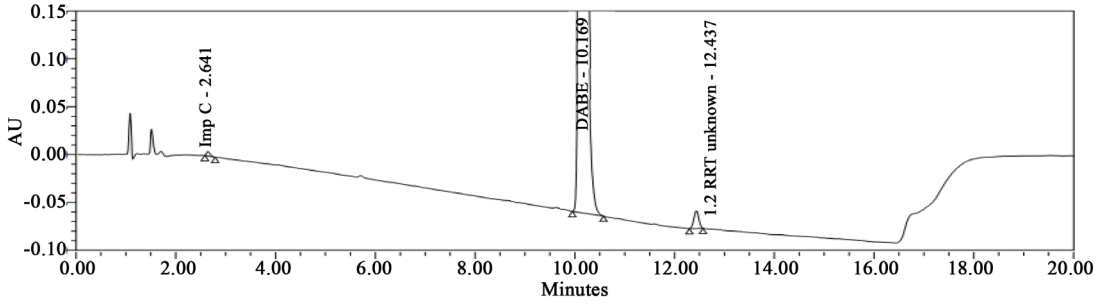
Imp E - 5.704
9.633
DABE - 10.166
1.2 RRT unknown - 12.435

(g)



(h)



(i)

Figure 16. Forced degradation (a) Acid 1NHCl; (b) Base 1N NaOH; (c) Thermal 70˚C; (d) Water, (e) Peroxide, (f). Humidity; (g) Sunlight; (h) UV light; (i) Unstressed.

Table 11. Peak details.

Compound	RT (min)	RRT	RRF	Resolution	Tailing factor
Imp C	2.642	0.26	1.26	-	1.1
Imp B	3.327	0.33	1.42	4.57	1.2
Imp E	5.724	0.56	1.01	15.75	1.2
DABE	10.170	1.00	1.00	25.68	1.2
Imp H	11.503	1.13	1.06	6.78	1.2

Table 12. Specificity.

Stress condition (Degradation)	% of impurities formed					% of degradation	Peak purity[#]	
	Imp C	Imp B	Imp E	Imp H	Unknown 1.2 RRT		Purity angle	Purity threshold
As such sample	0.13	ND*	ND	ND	0.74	0.88	0.783	1.616
Acid (1N HCl, 30 m, 60˚C)	ND	ND	9.11	ND	2.91	12.27	0.495	1.217
Base (1N NaOH, 15 m, 60˚C)	0.13	ND	21.55	ND	1.21	23.11	0.878	2.151
Thermal (48 hrs, 70˚C)	0.18	ND	0.07	ND	0.71	1.07	0.731	1.583
Water (3 hrs, 75˚C)	0.6	ND	0.49	ND	28.18	29.71	0.410	0.603
Oxidation (3%H_2O_2, 2 hrs, 75˚C)	3.44	ND	0.74	ND	23.17	27.9	0.295	0.555
Sunlight 1.2 million Lux hours	0.13	ND	0.05	ND	0.45	0.68	0.734	1.817
UV light (200 wats/m^2)	0.13	ND	ND	ND	0.45	0.58	0.526	2.204
Humidity 7 days, 90% RH/25˚C	0.15	ND	0.06	ND	1.04	1.27	0.824	1.564

*ND-Not detected; #Peak purity numbers represented as per waters Empower software algorithm. Peak is pure only if purity angle is less than purity threshold.

The %RSD of DABE during the assay method precision and intermediate precision was 0.7% and 0.9% respectively. The %RSD of replicate determination% area was found to be <5% in both precision and intermediate precision, which indicates that the method is precise and the data of precision studies are shown in **Table 13**. The recovery of DABE from drug product ranged from 97.6 to 100.8%. The results obtained from the recovery study were found within the range of 90% to 110% (LOQ to 200%), indicates that method is accurate and data for the same was shown in **Table 14** and **Table 15**. Sensitivity of the method was verified and the method was found to be linear, accurate and precise at limit of quantification and the data of LOD & LOQ studies are given in **Table 13**. The linearity calibration plot for the assay method was obtained over the calibration ranges tested, and the correlation coefficient obtained was >0.999. The calibration curve of all impurities were obtained by plotting the peak area of individual impurity versus concentration over the range of about LOQ- 5 µg/mL and were found to be linear (r = 0.999). The data of regression analysis of the calibration curves are shown in **Table 13**. No significant changes in the amounts of the three impurities were observed during the solution stability (on the bench top) and mobile phase experiments when performed by the related substances method but a significant conversion of DABE to 1.2 RRT unknown impurity is observed. So test solution and standard solution are stable only 6 hrs for both Assay and RS method.

Table 13. Regression and precision data.

Parameter	DABE	Imp C	Imp B	Imp E	Imp H
LOD (µg/mL)	0.12	0.14	0.13	0.15	0.16
LOQ (µg/mL)	0.41	0.44	0.42	0.46	0.5
Correlation coefficient	0.9997	0.9999	0.9999	0.9999	0.9999
% of Y-intercept	1.0	0.2	1.6	2.0	0.8
Precision (%RSD)	0.6%	0.9%	0.5 %	0.8 %	0.8%
Intermediate precision (%RSD)	0.8%	0.7%	1.2 %	1.1 %	0.9
Precision at LOQ(%RSD)	1.2 %	2.2%	1.7 %	1.8 %	2.4%

Table 14. Accuracy of impurities at sample test concentration of impurities.

Amount spiked*	% of Recovery†			
	Imp C	Imp B	Imp E	Imp H
LOQ	99.4 ± 1.7	98.7± 2.4	98.8 ± 1.9	98.8 ± 1.9
50%	98.5 ± 1.3	98.2 ± 0.8	100.6 ± 1.5	100.1 ± 1.1
100%	98.6 ± 0.8	98.4 ± 0.4	99.2 ± 1.3	99.1 ± 1.2
150%	98.2 ± 1.1	98.7 ± 0.8	97.4 ± 1.6	97.9 ± 1.5
200%	100.1 ± 0.4	98.8 ± 1.9	99.9 ± 1.4	100.4 ± 1.2

*Amount of three impurities spiked with respect to 0.5% specification level individually to DABE. †Mean ± %RSD for three determinations at each level.

Table 15. Accuracy of DABE at sample test concentration of assay.

Amount spiked*	% of Recovery†
	DABE
50%	99.2 ± 0.9
75%	99.1 ± 1.3
100%	99.5 ± 1.1
125%	99.2 ± 1.2
150%	98.8 ± 0.8

†Mean ± %RSD for three determinations at each level.

6.1. Isolation and Identification of DABE Water Degradation Product

During forced degradation studies of DABE capsules observed one major unknown degradant impurity along with other known impurities. It is forming majorly in water degradation study. The same impurity was enhanced during drug substance water degradation. This was confirmed with relative retention time, spectral match and LC-MS analysis. The degradation product in water hydrolysis (RRT~1.2) was prepared by using DABE drug substance. The DABE drug substance was dissolved in 30% acetonitrile and kept in reflux at 75˚C for 48 hours. Colorless crystals are formed. The separation of product from the reaction medium is due to its insolubility in water. The sample was concentrated using the rota-evaporator to remove solvents then lyophilized using a freeze dryer to obtain a white powder with >85% purity.

6.2. Structure Elucidation of Degradation Product

LC-MS and HPLC study were carried out to determine m/z values and RRT's respectively. The major degradation product formed under water hydrolysis using Agilent 1100 series liquid chromatography system coupled with 6400 series triple quadrupole mass spectrometer with suitable volatile mobile phase that contained acetonitrile and ammonium acetate in water in a ratio of 50:50 (v/v). The m/z value obtained for the degradation product resolving at 1.2 (RRT) in ESI positive mode was 629.7 $[M+H]^+$ and corresponds to the molecular weight of 628.3.

The IR spectra performed on dispersion KBr of DABE show the following absorption bands, 3500 - 3300 (one broad N-H stretching of enamine and one broad N-H stretching band of amine) which is a clear indication of enamine group, 2940 - 2850 (CH, CH2, and CH3 stretching), 1715 (C=O Saturated carbonyl group), 1666 (C=O conjugation); whereas RRT~1.2 water degraded impurity show the following absorption bands 3344 (broad N-H stretching of amine), which is a clear indication for the absence of enamine group , 2950 - 2850 (CH, CH2, and CH3 stretching), 1759 - 1732 (C=O groups of amide and esters) and 1660 (C=O conjugation).

The ^{1}H NMR spectrum of DABE shows two broad singlets at δ 10.64 and δ 11.88 which indicate the presence of enamine functionality in DABE. Whereas the ^{1}H NMR spectrum of water degraded impurity shows no such peaks clearly indicating the hydrolysis of enamine to amide.^{1}H NMR assignments for unknown impurity and DABE are shown in **Table 16**. The ^{13}C NMR spectra of DABE and the impurity have no difference indicating that the basic skeleton of DABE is not disturbed during degradation.

Table 16. H NMR assignments for unknown impurity and DABE.

Position	Unknown impurity	DABE
	δ H	δ H
1	0.87 (3H, t)	0.87 (3H, t)
2	1.09 (3H, t)	1.09 (3H, t)
3	1.32 (6H, m)	1.34 (6H, m)
4	1.60 (2H, m)	1.65 (2H, m)
5	2.68 (2H, t)	2.68 (2H, t)
6	3.76 (3H, s)	3.78 (3H, s)
7	3.95 (2H, m)	3.98 (2H, m)
8	4.08 (2H, t)	
9	4.22 (2H, t)	4.22 (4H, m)
10	4.60 (2H, d)	4.69 (2H, d)
11	6.73 (2H, d)	
12	6.87-7.16 (4H, m)	6.85 - 7.66 (11H, m)
13	7.39-7.80 (5H, m)	
14-NH	8.38 (1H, d)	8.39 (1H, d)
15-NH	10.45 (1H, s)	10.02 (1H, s)
16-NH2	-	10.64 - 11.88 (2H, broad singlets)

6.3. Degradation Pathway Explanation

As per forced degradation data, two major degradants are forming. One is impurity E another one is 1.2 RRT unknown impurity. It observed significant degradation in Acid and Base hydrolysis leading to form Impurity E.

6.4. Unknown Impurity (RRT1.2) Formation in Water Hydrolysis

Generally DABE exists in the form of Enamine. During the forced degradation of DABE in water medium at 70˚C - 75˚C, amine of enamine abstracts the proton from water and form quaternary ammonium salt (**1**). To get stabilize this intermediate, it abstracts lone pair of electrons to form ammonia gas and the resulting carbonium ion is stabilized by taking hydroxyl group from water to form an intermediate (**2**). This intermediate (**2**) always exists in keto-enol tautomer. The driving force for this reaction is the formation of ammonia gas. The separation of product from the reaction medium is due to its insolubility in water. The possible mechanism presented in **Figure 17**.

6.5. Impurity E (Dabigatran Etexilate Acid) formation in Acid hydrolysis

The ester group of Dabigatran takes a proton (a hydrogen ion) from acid to form hydroxonium ion and it leads to form carbonium ion (An intermediate). The carbonium ion gets stabilized by accepting hydroxyl group to form an intermediate, which finally forms DABE acid by the loss of ethanol. The possible mechanism presented in **Figure 18**.

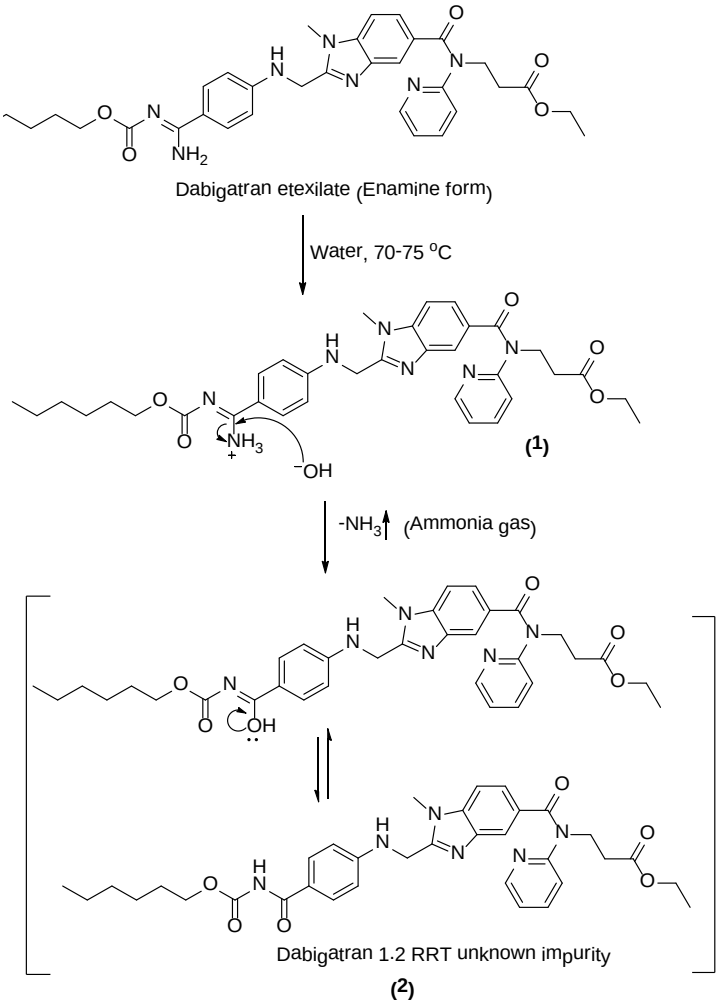


Figure 17. Possible mechanism for 1.2 RRT unknown impurity formation.

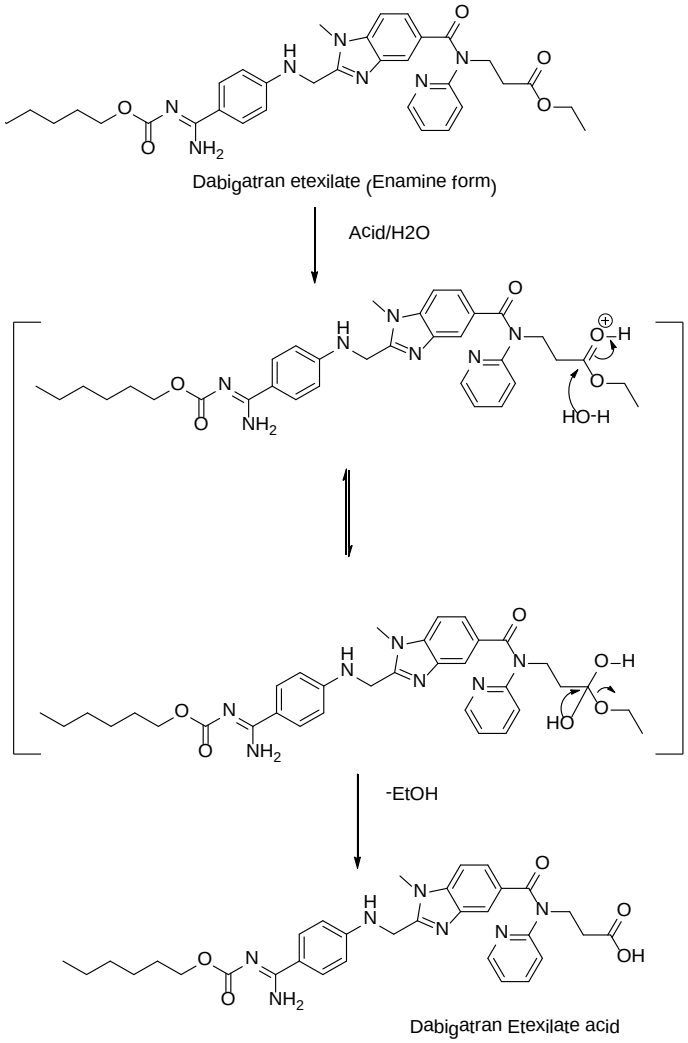


Figure 18. Possible mechanism for Impurity-E formation in Acid condition.

6.6. Impurity E (Dabigatran Etexilate Acid) Formation in Base Hydrolysis

The hydroxide nucleophiles of base attack the electrophilic carbon of the ester of DABE to form the tetrahedral intermediate. The intermediate stabilizes by the loss of ethoxide to form salt of acid. Upon acidification, finally the resulted product will be DABE acid. The possible mechanism presented in **Figure 19**.

7. Conclusion

A reversed-phase LC method development workflow based upon QbD principles has been designed and demonstrated. The workflow starts with understanding the method needs (ATP), followed by technique selection, method design, risk assessments to identify and DOEs to alleviate experimental risk factors. The approach leverages structural knowledge of the samples, automation, chemometric data reduction, and software-based decision support. This is a rapid and efficient approach and minimizes the amount of time that the analytical scientist spends performing method development and data analysis, while investigating a very broad scope of experimental variables. The end result is a robust chromatographic method with a well-understood MODR and control strategy. The resources spent developing a method via an AQbD philosophy are significantly less over the lifecycle of the method as compared to traditional approaches, especially when method transfer related issues are factored in. The gap identified in the current workflow is on the refinement and streamlining of the data processing parameters as well as interfacing with DOE statistic package to provide an automated QbD workflow

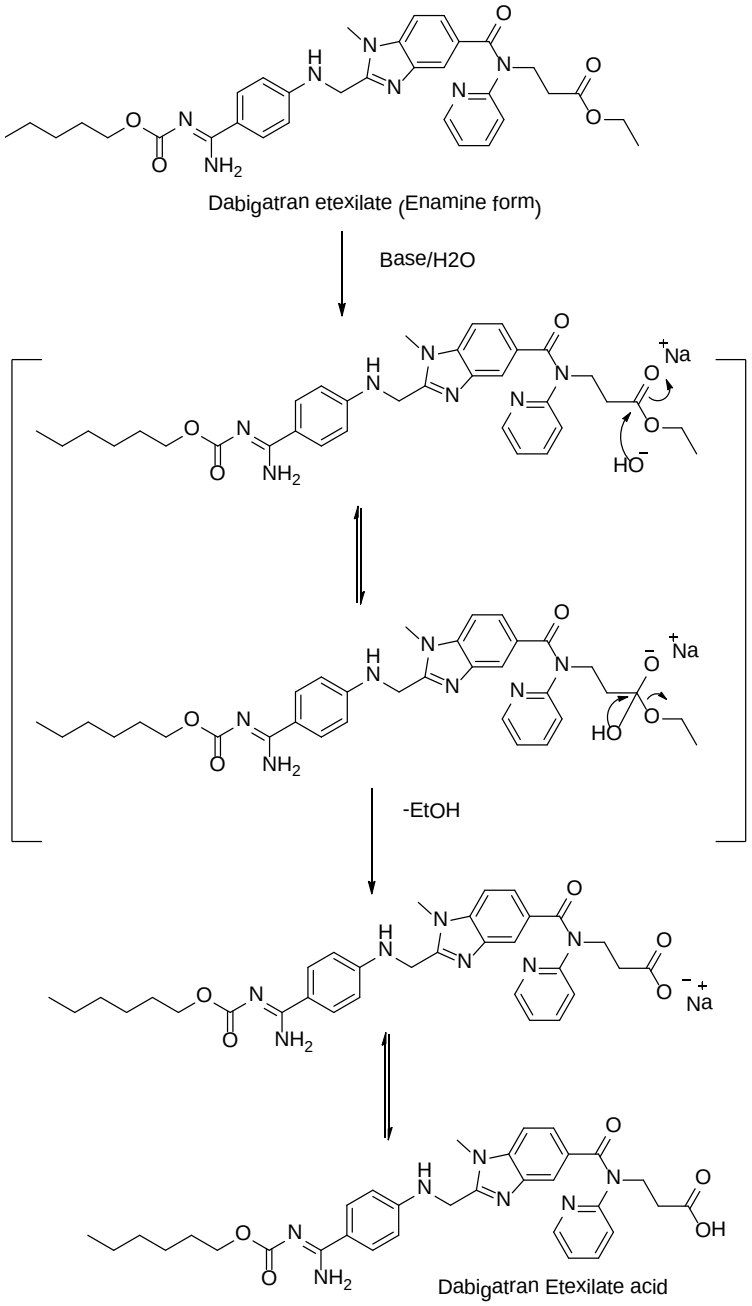


Figure 19. Possible mechanism for Impurity-E formation in Base condition.

from samples to MODR. The simple HPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the result obtained from analysis of two different formulations demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the HPLC method developed in this study make it suitable for quality control analysis of complex pharmaceutical preparation containing DABE and its impurities.

Acknowledgements

This article does not contain any studies with human and animal subjects performed by any of the authors. All

authors (H.R. Bapatu, R.K. Maram, W.H. Cho, P. V. B. Rao) declare that they have no conflict of interest. Authors express sincere thanks to the Managements of Dr. Reddys Laboratories, Hyderabad, India; Celltrion Chemical Research Institute, South Korea for their support and encouragement.

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