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Analytical Quality By Design For Development Of A Sensitive, Rapid And Stability Indicating Method For Estimation Of Impurities Of Dimenhydrinate In Its Pharmaceutical Formulation.

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ABSTRACT

The purpose of this study was to implement QbD (quality by design) principles to develop a simple, sensitive and rapid RP-UPLC (Reversed phase Ultra Performance Liquid Chromatography) method for the separation and quantification of Dimenhydrinate impurities in its dosage form, Dimenhydrinate ODT (Orally disintegrating tablets).The method was developed with predefined analytical target profile. The method employs XSelect HSS T3 (100*2.1mm, 1.8 µm) chromatographic column with Mobile phase A as mixture of Phosphate buffer pH 2.5: Acetonitrile (65:35) and Mobile phase B, a mixture of Phosphate buffer pH 2.5: Methanol (5:95) in gradient run. The injection volume was 2µl with column temperature of 30ºC and working wavelength of 225nm. The composition of mobile phases and gradient program were evaluated through DOE. Main effects of percentage Acetonitrile in Mobile phase A, percentage Methanol in Mobile phase B, gradient steepness and their interaction effects on critical quality attributes (CQA) were established. The design space for the method was established through CCD (Central Composite Design) statistical model. The QbD compliant method was successfully developed and validated for Specificity, Linearity, Accuracy, Repeatability, Range, Limit of detection and quantitation. The method was proved for its stability indicating nature by forced degradation studies.

Keywords: Quality by design, Diphenhydramine, 8-Chlorotheophylline, Design of experimentation

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INTRODUCTION

Quality, Safety and Efficacy are the three fundamental requirements of any pharmaceutical product. The efficacy of the pharmaceutical product shall be determined by bioavailability and bioequivalence studies. The safety of a drug product shall be determined by pharmacological and toxicological studies. As some of the impurities are known to be toxic even at low concentrations, the toxicological properties of drug product are not only determined by the toxicological properties of drug substance and also by impurities that it contains. Webster's dictionary defines impurity as something which is impure or makes something else impure. As impurities do not have any intended pharmacological activity, their levels must be monitored and controlled through a well-defined and scientifically acceptable specifications and analytical methods. The quality of pharmaceutical drug product is determined by measuring its compliance to the pre-established and well defined specifications. The analytical methods used in quantification of impurities in pharmaceutical products during development stage of drug product or in quality control should be able to quantify impurities accurately even at trace levels. ICH Q3B (R2) guideline [1] states that the analytical method used for quantification of impurities in pharmaceutical products "have been validated and are suitable for detection and quantitation of degradation products. As appropriate, the validation activity should include samples stored under relevant stress conditions like light, heat, humidity, acid/base hydrolysis, and oxidation". Also, ICH Q8 (R2) [2] (Pharmaceutical development) talks about Risk assessment tool "Ishikawa Diagram" where in Analytical method is a variable which can have impact on desired quality attributes of product.

The principles of QbD as a basis for product development have been well defined by ICH Q8 (R2). Pharmaceutical industries are in communication with United States Food And Drug Administration (USFDA) for developing the products using QbD via pilot programs. However, **this** guideline does not specifically mention the use of QbD concept for analytical methods. Since the analytical method is very critical factor as per "Ishikawa Diagram", it becomes necessary that these concepts are equally important and applicable to analytical method developments in order to have proper control, reproducible results and to meet current regulatory requirements. The drawbacks for an analytical method developed only by OFAT (One factor at a time) approach have been discussed in detail [3]. The utilization of QbD as a basis for analytical method development gives a thorough understanding on method and method control parameters and this will in turn help in minimizing frequent and repeated OOS (Out of specification), OOT (Out of trend) results, post approval changes and issues during inter-laboratory method transfers. Also, quality of product can be best measured by following a set of instructions (compliance) that are shown to repeatedly give the same product and is supported by analytical testing [4]. QbD along with Quality risk management (ICH Q9, Quality Risk Management) [5] and an appropriate Pharmaceutical quality system (ICH Q10, Pharmaceutical Quality System) [6], provides opportunities to develop science and risk-based regulatory approaches. Application of ICH Q8, Q9 and Q10 for analytical method development requires a well-planned evaluation on purpose of method development and routine use based on detailed understanding of science supporting the analytical methodology.

The application of QbD for analytical methods can be broadly classified in to two parts [4]. The first one is the analytical target profile (ATP) which defines the objective of the measurement and forms the basis for development of the initial method. The second concept addresses on how QbD steps and approaches can be applied to strategize, improve and lifecycle management of an analytical method as applicable for pharmaceutical formulations.

Design of experimentation (DOE) is an integral part of QbD wherein multiple experimental factors can be varied simultaneously through statistical factorial designs to study their main and interaction effects. For related substances method development by chromatography, as analyte molecules in the drug product will have different physico chemical factors, Even the well-constructed factorial design may not give accurate (give false) results in terms of selectivity changes wrt stationary phase selection, pH and Organic modifier in the mobile phase. Thus, a wary and well- planned scientific approach is necessary for designing the DOE experimentation and using QbD concept for analytical method development. The focus of this paper is on implementation of QbD concept as a system for related substances method development for Dimenhydrinate ODT dosage form.

To provide guarantee of future variations, QbD talks about Design space (DS). Mathematically, design space is defined as $\mathrm{DS} = \{\mathcal{X}\mathrm{o}\epsilon\chi|\mathrm{Eo}[\mathrm{P}(\mathrm{CQAs} \in \Lambda)]|\mathrm{X} = \chi, \theta] \geq \pi\}$. In other words, we look for a region in an

experimental domain (often called knowledge space) where the expected probability that CQAs are within specifications. With this knowledge, extensive literature search activity was undertaken to comprehend the use of QbD concept for analytical method development for impurities of Dimenhydrinate ODT. There are publications [7-20] which focus on analytical method development using QbD Concept for dosage forms containing different drug substances. Also, literature search reveled that there are few publications [21-24] discuss about the assay of Dimenhydrinate with other drugs in a combination dosage form. There are very few publications [25-27] discuss on quantification of impurities of Dimenhydrinate in its pharmaceutical formulations. European pharmacopoeia [25] and Duge, Eger [26] method for the quantification of related substances of Dimenhydrinate (DMH) are comparable in terms of eluent A, eluent B, Mobile phase modifiers like Triethylamine (TEA), Acetonitrile and elution order of impurities. They differ in terms of the gradient time program. The retention factor "k-value" for these methods is 2 to 54 and 4.4 to 18 minutes respectively, against the recommended k-value of between 2 and 10. Methods with high retention factor are not friendly for the routine quality control and product development purposes. Also, these methods are developed based on one factor at a time (OFAT) approach and do not have sufficient data to cater the routine laboratory variations which can impact the separation between the critical band pairs like separation between Diphenhydramine (DPH) and Impurity F. The run time for method is at 45 minutes which limits throughput of the equipment, consumption of more solvents, chemicals and laboratory resources. Further, there is no flexibility in these methods to cater the requirements of any post approval changes and Quality Target Product Profile (QTPP) changes. The optimization of chromatographic methods for quantification of impurities is often complex due to wide range of parameters and factors which can influence on the selectivity and sensitivity of the separations and such separations can be best optimized by QbD. Thus, it was thought worthwhile to develop a stability indicating UPLC method for estimation of impurities for Dimenhydrinate in Dimenhydrinate ODT using QbD principles with shorter run time and validate the same as per ICH Q2 (R1) guideline [28].

Dimenhydrinate (DMH) chemically known as 1H-Purine-2,6-dione, 8-chloro-3,7-dihydro-1,3-dimethylcompound with 2-(diphenylmethoxy)-N,N-dimethylethanamine (1:1). Dimenhydrinate actually a combination of two drugs; DPH and (8-Chlorotheophylline) CT.DPH is the primary constituent of Dimenhydrinate and is responsible for causing the primary effect. It is an over-the-counter antihistamine drug used for the treatment of nausea, vomiting, and dizziness caused by motion sickness. As per USP (United States Pharmacopoeia), by weight DMH is between 53.0% to 55.5% of DPH and 44.0 to 47.0% CT, a chlorinated derivative of Theophylline, which counteracts the drowsiness. The structures of DPH, CT and impurities are as given in Figure 1

EXPERIMENTAL

Standards and reagents

Standards of high purity for DMH, DPH and CT were provided by SPI Pharma Inc, India. The purity of these standards was determined by HPLC and was found to be 99.4%, 98.9% and 98.3% respectively. Sodium hydroxide, Hydrochloric acid, Hydrogen Peroxide, Orthophosphoric acid, Triethylamine (TEA) of AR grade and Acetonitrile & Methanol (HPLC grade) were purchased from Rankem, India. The impurity standards (Benzhydrol, Benzophenone and Theophylline) were provided by SPI Pharma Inc, India. Impurity F of Dimenhydrinate was purchased from European pharmacopoeia (EP). Dimenhydrinate ODT 50 mg (Test product) with batch number 078/E026A, 078/E026B and placebo were manufactured at SPI Pharma Inc., India branch.

Instrumentation

The Chromatographic column XSelect HSS T3 (100*2.1mm, 1.8 µm) was purchased from Waters Corporation (Milford, MA, USA). Analytical method development, Quantitative analysis and Method validation **parameters** were performed on Waters Acquity UPLC (Binary) system equipped with PDA Detector. The UPLC instrument was operated through Waters Empower3 Software. The pH measurements were done using pH meter SevenEasy Model (Mettler Toledo, Columbus, OH, USA).The statistical treatment of data was done using Design expert software version 8.0.7.1(Stat ease, USA). The water used for experimentation was from Merck Millipore, Q pack water purification system.

Methods

The definition of QbD as per ICH Q8 (R2) is "A systematic approach to development that begins with predefined objectives and emphasizes product ,process understanding and process control based on sound science and quality risk management" [5]. For the method development purpose, this definition can be interpreted as to define the criteria for the acceptable separation i.e Analytical target profile (ATP) based on the QTPP , CQA's of the drug product, chemical nature of analyte molecule, application of the method and control based on scientific rationale.

As the emphasis was on development of a flexible and high throughput method to quantify the impurities of Dimenhydrinate in its pharmaceutical formulation containing a matrix of functional excipients, sweetner, flavor and color. The analytical target profile of method was defined as; The method should be specific and stability indicating with all degradation impurities well resolved from known impurities, CT and DPH should be quantifiable at levels as low as 0.03%. The precision of the method must be such that the % RSD (Relative standard deviation) for 6 independent sample preparations must be ≤ 10.0%. The accuracy of method must be such that the recovery values for each individual known impurity must be within the range of 85.0 ±15.0% of true values and must be with linearity correlation coefficient and regression coefficient of NLT(Not less than) 0.99 for all impurities. The design space for method must be such that the critical band pairs have acceptable resolution (R_s) to meet the routine quality control variations. With these predefined analytical target profile, separations were recorded at 225nm.To achieve the defined ATP, following experiments were performed.

Optimization of chromatographic conditions

Selection of a column stationary phase for separation is an important step in method development. As the sample contains highly polar compound (Theophylline) and a compound with mid polar properties (DPH) and nonpolar compound like Benzophenone, the stationary phase selection was done to retain Theophylline and to have optimum retention for DPH. Waters XSelect HSS T3 2.1*100mm, 1.8µm stationary phase was selected for the separation as this stationary phase comprises of high strength silica which can withstand high pressures inherent in UPLC separations. T3 bonding utilizes a trifunctional C_{18} alkyl phase bonded at a ligand density that encourages polar compound retention and high aqueous mobile-phase compatibility even at acidic pH's like 2.5. As the T3 endcapping is much more effective than traditional end-capping techniques like trimethylsilane (TMS) which helps in overcoming the inherent peak tailing issues of DPH peak. This exclusive combination of bonding and endcapping also enhances column performance, lifetime, loading capacity, selectivity and stability of stationary phase. The selectivity changes in terms of resolution and retention time of peaks were used in screening organic portion of the mobile phase. Both Acetonitrile and Methanol were evaluated as organic modifiers in mobile phase along with aqueous buffer. The mixture of buffer and Acetonitrile in the ratio of 1:1 was used as diluent.

Gradient program

The initial gradient run (t(min)/ %B:0/15 and 40/90 of Methanol) provided an estimate of % organic ratio and approximate retention range for impurities. The retention time for first Theophylline (TPH) and last peak (Benzophenone) were 0.76 and 7 minutes for which the difference ($t_{R\ Benzhydrol-}\ t_{R\ Theophylline)}$ was $\Delta t_R = 6.24$. The impurity F and DPH peaks were co-eluted. Additional experiments were taken by modification of gradient program and change in type of modifier to get a clear base line and to study the selectivity changes.The gradient program was adjusted to minimize run time of method maintaining good resolution between the analyte peaks. Acetonitrile was selected in the Mobile phase A as organic modifier to have a good resolution between impurity F and DPH. Methanol was used as organic modifier in mobile phase B to reduce the run time and maintaining resolution (R_s) between Benzhydrol and Benzophenone more than 2.0.

Design of experimentation

Design of Experimentation (DOE) is an integral part of QbD concept to screen for vital few factors from trivial many factors causing influence on separation and to decide on acceptable level of the factors. It

was also used to study the effect of individual factors and their interaction effects. Three Critical process parameters (CPP) were recognized as having an impact on separation quality. They are the concentration of TEA in buffer (%v/v), Percentage of Acetonitrile (%v/v) in mobile phase A and Percentage of Methanol (%v/v) in mobile phase B. The range and units are presented in Table 1. The CQA's considered were retention time (RT) of Diphenhydramine (in minutes), Benzhydrol and Benzophenone peaks and resolution (R_s) between Impurity F and DPH peaks, Benzhydrol and Benzophenone peaks. A full factorial two level screening DOE with 8 runs was run and CQAs were recorded. The data was subjected for statistical treatment using Design-Expert software, version 8.0.7.1 (Stat-Ease, Inc., Minneapolis, MN, USA).The segmented gradient model program was used to have acceptable retention range for the peaks.

Screening DOE indicated the impact of CPP's on the overall separation and help in defining the ranges for the CQA's. Based on the learnings from the screening DOE, a full factorial two level design was constructed to further optimize the R_s between peaks and to have stable smooth base line with reduction in run time. The factorial design was constructed using design expert software for the factors and levels as given in Table 2. The resulting 8 runs were recorded in randomized manner in a UPLC and data was recorded.

In order to assess the impact of routine laboratory variations on quality of separation and to establish the design space for the method, a full factorial central composite design (CCD) model was constructed considering Acetonitrile (%v/v) in Mobile phase A and Methanol (%v/v) in Mobile phase B and gradient composition at 2.2 to 3.8 minutes of gradient program. CCD enables estimation of regression parameters to fit a second-degree polynomial regression model to a response. In order to develop correlation, CCD requires three types of experiments, *i.e.*, factorial trials, axial trials and center point trials. To establish the design space and to determine the working point, the central composite design was run at 3 levels with 6 repetitive centre points, 4 axial points. Totally 20 runs were recorded and the data was used for statistical treatment. The experimental design is as given in Table 3. The working point and design space for method were selected based on CCD data interpretation. The working point obtained from the data treatment was verified.

The newly developed QbD compliant method was validated as per ICH Q2 (R1) guideline

Specificity

The specificity of method was demonstrated by checking the interference of sample matrix, blank and degradation impurities with DPH, CT and known impurities. The stability indicating nature of method was demonstrated by forced degradation studies. For acid degradation, 2ml of 1N HCl was added in to 50ml volumetric flask containg sample equivalent to 50 mg of DMH and kept at 80ºC on a water bath for 45 minutes. At the end of 45 minutes, the sample was taken out and cooled to room temperature. To this solution added 2ml of 1N NaOH to neutralize unreacted acid and made up to mark with diluent. For alkali degradation, 2ml of 1N NaOH was added in to 50ml volumetric flask containg sample equivalent to 50mg of DMH and kept at 80ºC on a water bath for 45 minutes. At the end of 45 minutes, sample was taken out and cooled to room temperature. To this solution, added 2ml of 1N HCl to neutralize unreacted base and made up to the mark with diluent. For oxidation degradation, 2ml of 3%v/v hydrogen peroxide solution was added in to 50ml volumetric flask containing sample equivalent to 50mg of DMH and kept at 80ºC on a water bath for 45 minutes. At the end of 45 minutes, sample was taken out and cooled to room temperature and made up to mark with diluent. In case of thermal degradation, sample was exposed to 80ºC for 22 hours in a hot air oven. At the end of 22 hours, sample was taken out and cooled to room temperature. Sample quantity equivalent to 50mg of DMH was weighed in to 50ml volumetric flask, added 20ml of diluent and dissolved. Made upto mark with diluent.For neutral degradation, 2ml of water was added in to 50ml volumetric flask containg sample equivalent to 50mg of DMH and kept at 80ºC on a water bath for 45 minutes. At the end of 45 minutes, sample was taken out and cooled to room temperature. The sample was added with 20 ml of diluent , made up to the mark with diluent. All the sample preparations were filtered through 0.22 µ filter and injected on to UPLC. The peak purity values for DPH, CT and all known impuritiy peaks were checked in each of the stress conditions.Individual known impurity solutions were prepared at 2 µg/ml and injected to establish the RT for each of them.

Accuracy

The accuracy was determined by spiking placebo based test solution (1000 µg/ml) with known amount of impurities. Impurities were weighed to prepare a common stock solution. From the stock solution the sample was spiked at 0.1,0.2 and 0.6% concentrations in triplicate. Similarly, accuracy of DPH and CT were performed in placebo matrix at 1, 2.5 and 8% concentrations in triplicate and recoveries were reported. (Table 10).

Linearity

Linearity test solutions were prepared at seven levels of 0.02, 0.04, 0.1, 0.2, 0.4, 0.6 and 1.1 % concentration for each impurity. The calibration curves were drawn by plotting the average analyte peak area against the concentration in µg/ml. In the similar manner, the linearity of DPH and CT were also established at seven concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 0.7 and 1.2%. The results of correlation (r) and regression coefficient (R^2) are as shown in Table 10.

Repeatability

The repeatability parameter was evaluated by analyzing six individual determinations of test (540 µg/ml DPH and 460 µg/ml of CT) with spiked impurities at 2µg/ml. For % impurity values obtained (*n=6*), standard deviation (SD) and % relative standard deviations were calculated (% RSD). The results are as given in Table 10.

Range

Range of method was determined from the linearity, accuracy and repeatability studies data.

RESULTS AND DISCUSSION

Selection of working pH for the method was an important step in obtaining required selectivity. The pKa values were 9.12 for DPH and 5.2, 8.2 for CT. Based these pKa values, decided to work in the acidic pH. Working in the acidic pH like pH 2.5 will also eliminate the peak tailing issues for the analyte peaks. A detector wavelength of 225nm was selected as all impurities, DPH and CT had optimum responses. As the analytes in sample mixture had a wide range of the polarities with TPH being highly hydrophilic and Benzophenone being hydrophobic, the need for gradient run was assessed using mobile phase A as 35:65 mixture of Phosphate buffer pH 2.5: Acetonitrile and Mobile phase B as 5:95 mixture of Phosphate buffer pH2.5: Methanol.

Study of main and interaction effects

Significant factor for Resolution

The main effect and interaction effect plots for concentration of Acetonitrile (% v/v) in Mobile phase A, concentration of Methanol (% v/v) in Mobile phase B and concentration of TEA (% v/v) in buffer on R_s between DPH and impurity F , RT's of DPH, Benzhydrol and Benzophenone were studied in the screening DOE. The screening experiments show that the concentration of Acetonitrile has direct impact on R_s between DPH and Impurity F. Also, The RT's of DPH and Benzhydrol were affected by concentration of Acetonitrile in Mobile phase A. Interestingly RT of Benzophenone was affected by both Acetonitrile and Methanol concentration in Mobile phase A and B.TEA did not had any impact on CQA's at the specified concentration. Based on screening DOE observations, a two level full factorial design was constructed by reducing the range of CPP's. The data obtained was subjected to statistical treatment using ANOVA. ANOVA is a statistical method based on the F-test that assesses the significance of the experimental results. It involves subdividing the total variation of the data set into component parts. The F-value in ANOVA table is a ratio of model mean square (MS) to the appropriate mean square. The larger is their ratio, larger the F-value and more likely that variance contributed by model is significantly larger than random error. The p-value for test is conducted using an F-statistic. If the F-ratio lies near the tail of F-distribution, probability of a larger F is small and variance ratio is judged to be significant.

The effect of % Acetonitrile and gradient time on the RT of DPH and resolution between DPH and Impurity F is given in the main effects plot in ANOVA Table 4 and Table 5.

Table 4 and Table 5 depicts the main effects of Acetonitrile concentration and gradient time on the RT of DPH and main effect of Acetonitrile on the R_s between DPH and Impurity F. With the F value of 34.05 and p-value for the model is <0.05 indicating that the model terms are significant. The percentage of Acetonitrile in Mobile phase A has very high impact on the RT of the DPH peak. With the increase in Acetonitrile concentration in mobile phase A, the RT of the DPH decreases. The second significant factor affecting the RT is gradient time. In Table 5 with F value of 2187.00, there is only 0.01% chance that the model F value of this large can occur because of noise. Interestingly only concentration of Acetonitrile has impact on the R_s between DPH and impurity F. The composition of Mobile phase B and gradient program does not have any impact on RT of DPH and R_s between DPH and Impurity F.

The ANOVA data for RT of Benzhydrol and Benzophenone are given in Table 6 and Table 7. In Table 6, The model F value of 135.30 and p-value of <0.05 indicates that the model is significant. The gradient time followed by % Acetonitrile have major impact on the RT of Benzhydrol. With the increase in the concentration of Acetonitrile, the RT of Benzhydrol decreases. In Table 7, the model F value of 662.22 and p-value of <0.05 indicates that the model is significant. The % Acetonitrile, gradient time and % Methanol in Mobile phase B have impact on the RT of Benzophenone. The highest impact comes from the gradient time. Interestingly there is no interaction effects observed for all the CQA's considered.

CCD Data treatment for Resolution between DPH and Impurity F

The statistical values are presented in Table 8. F-value of the model for R_s between Impurity F and DPH is 7.56. With p-value falling less than 0.05 indicating that the model is significant. The "Pred R-Squared" of 0.0148, is not as close to "Adj R-Squared" of 0.7564 as one might normally expect. This indicate a large block effect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 8.366 indicates an adequate signal. This model can be used to navigate the design space. The 3D Numerical plot for resolution between Impurity F and DPH was constructed with two factors. Each point in plot indicates the response value at that particular value of factors.(Figure 2).

CCD Data treatment for Resolution between Benzhydrol and Benzophenone

The statistical values are as presented in Table 9. F-value of the model for R_s between Benzhydrol and Benzophenone is 6.72. With p-value falling less than 0.05 indicate that the model is significant. The "Pred R-Squared" of 0.3860 is not as close to "Adj R-Squared" of 0.7305 as one might normally expect. This indicate a large block effect. "Adeq Precision" value of 7.927 indicates the adequate signal. This model can be used to navigate the design space.

The 3D Numerical plot for R_s between Benzhydrol and Benzophenone was constructed with two factors. Each point in plot indicates the response value at that particular value of factors(Figure 3).

The combined 3D contour covering all CQA's is as presented in Figure 4. Figure 4 clearly depicts the value of each CQA at each point of concentration of Acetonitrile in Mobile phase A and Methanol in Mobile phase B.

In order to have repeatable system suitability criteria for analysis of development samples and for quality control purposes, the working point in design space was selected by statistical treatment of the data. The resulting 21 run conditions had desirability of 1.000. Considering the practicality of predicted conditions, solution 17 was selected for verification. The conditions were verified experimentally by fresh preparation of mobile phases and sample solutions. The experimentally obtained values were compared with predicted values and found that there is an excellent correlation between predicted and experimentally obtained values.

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Method Validation

Specificity

DPH, CT and all known impurity peaks were well resolved from blank, sample matrix and degradation impurities. In forced degradation; For acidic condition, DPH undergoes hydrolysis at ether linkage resulting in the formation of Benzyhydrol and 2-(dimethylamino) ethanol as degradation products along with unknown impurities. The percentage degradation observed is 11.4%. In peroxide condition, DPH is susceptable to oxidation resulting in the formation of Impurity F,Toluene, Benzhydrol,Benzophenone, Benzyl alcohol and other phenolic compounds as degradation products. The percentage degradation observed is at 13.8%. Also, CT undergoes oxidation to form Theophylline as major degrdation impurity.In alkali, thermal and neutral stress conditions; DMH is relatively stable. CT is stable in comparison to DPH in all the degradation conditions. In all degrdation samples, DPH, CT and all known impurities are well separated from each other and degradation impurities. The peak purity angle is less than the purity threshold indicating that the peaks are pure. The specimen chromatograms for degradation conditions are presented in Figure 5.

Accuracy

The recovery values obtained at each level were characterized by relative standard deviation. The values are presented in Table 10. The mean % recovery values (n= 9) for impurities and drug were within the range of 90.6±3.4 to 100.5±3.5 (mean± SD), indicating that the method was accurate.

Linearity

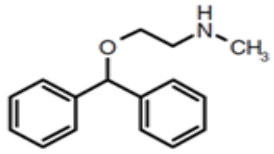
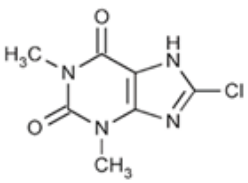
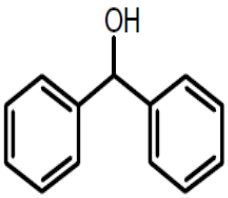
Results of correlation co efficient and regression co efficient are as shown in Table 10. The calibration curve was constructed using concentration (µg/ml) on x-axis and area response on y-axis. The calibration curve equation is y=mx+c, where y represents analyte peak area and x represents analytes concentration in µg/ml. The mean equation of calibration curve (n=7) obtained for each analyte peak were calculated. The method was found to be linear with the acceptable correlation and regression coefficient values.

Precision

The precision of method was expressed as percentage relative standard deviation (%RSD) value obtained for content of impurities in each sample preparation. The mean % RSD values for each impurity for 6 sample preparations is given in Table 10. These results confirm the higher precision of the method.

Range

The method was found to be linear for quantification of impurities from 0.2 µg/ml to 11 µg/ml. The accuracy for impurities was proved from 0.3 µg/ml (LOQ) to 6 µg/ml. The %RSD values for replicate preparation of sample solution spiked with impurities are satisfactory. Hence range of method proved to be from LOQ(0.3 µg/ml) to 6 µg/ml.

		
Diphenhydramine	**8-Chlorotheophylline**	**Benzhydrol**

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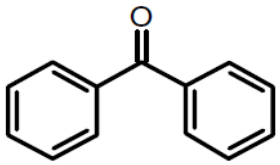	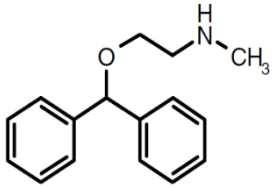	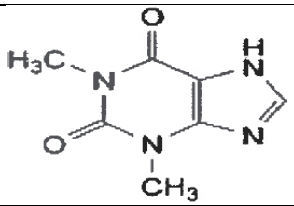
Benzophenone	**Impurity F**	**Theophylline**

Figure 1: Structure of a) Diphenhydramine b) 8-Chlorotheophylline c) Theophylline d) Impurity F e) Benzhydrol f) Benzophenone

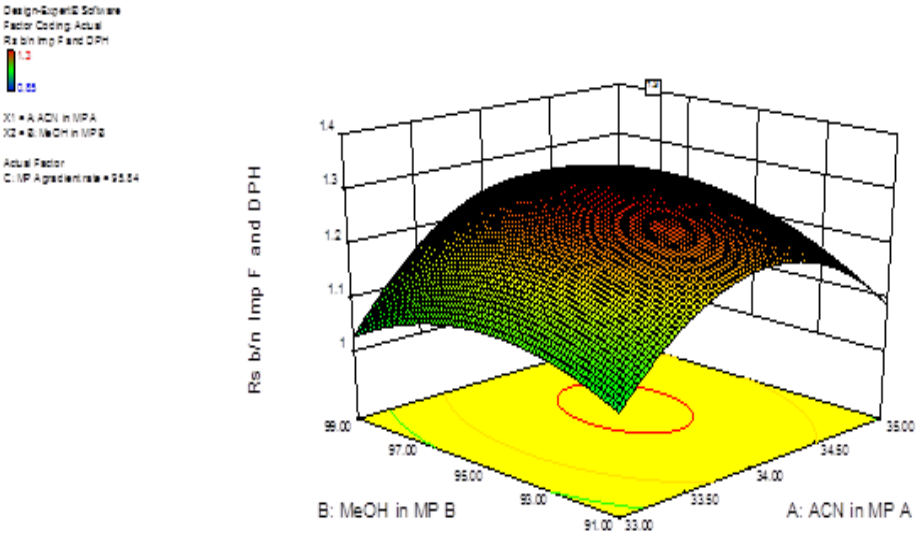

Figure 2: 3D Numerical plot for R_s between Impurity F and DPH

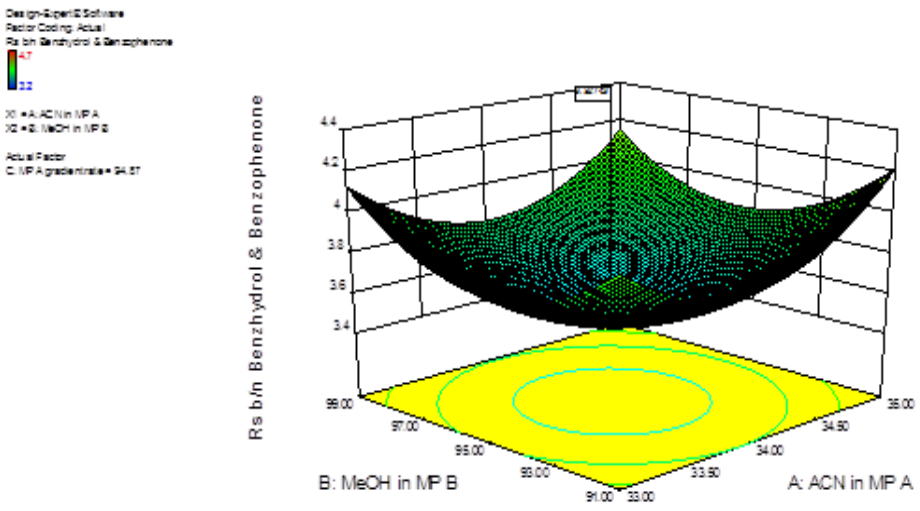

Figure 3: 3D Numerical plot for R_s between Benzhydrl and Benzophenone

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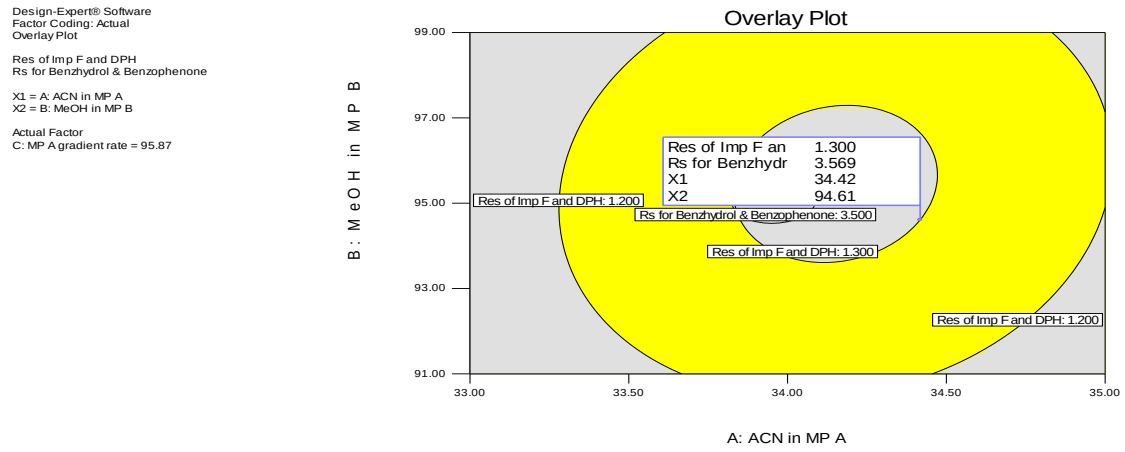
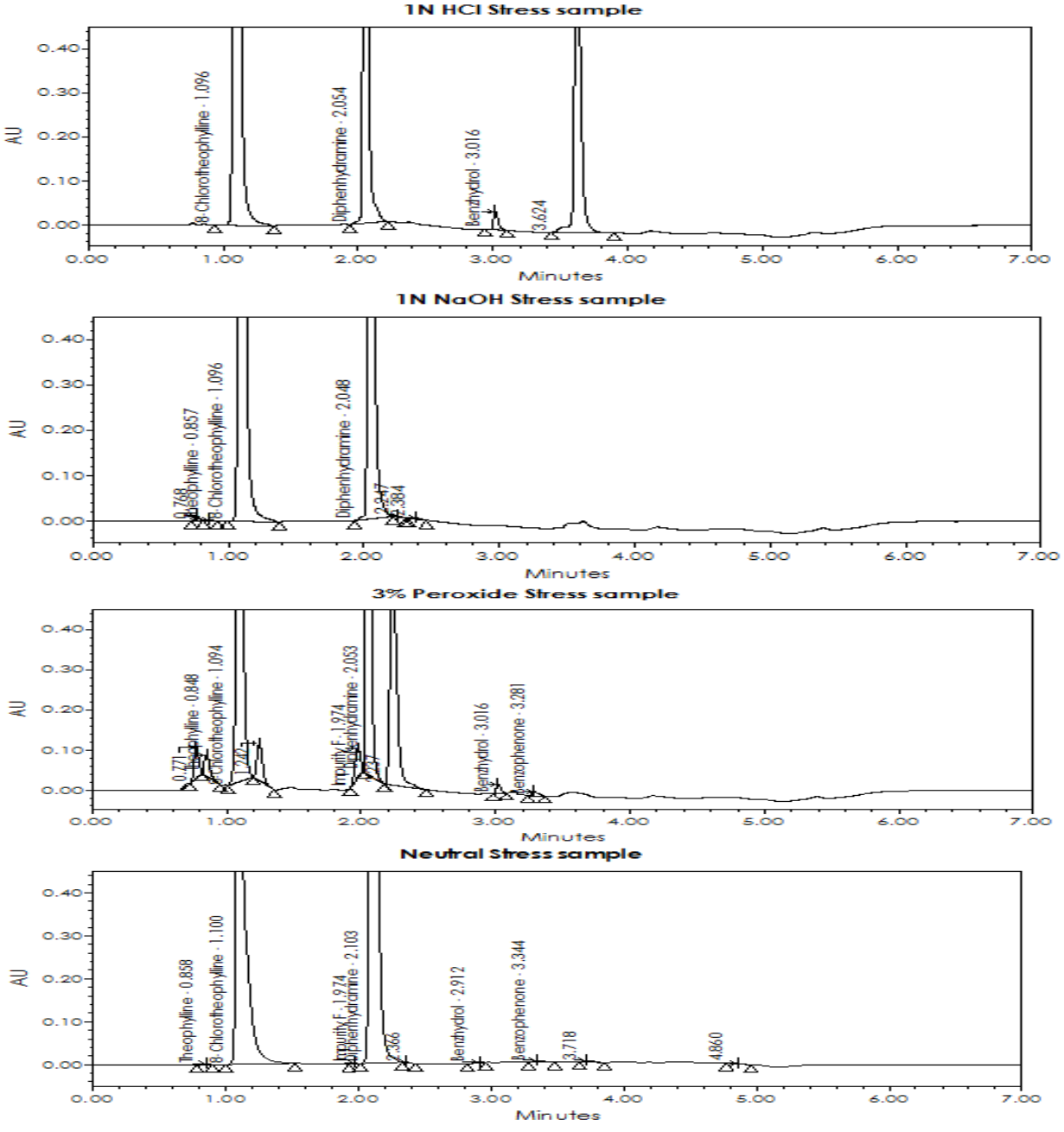


Figure 4: Combined 3D plot covering all the CQA's of CCD



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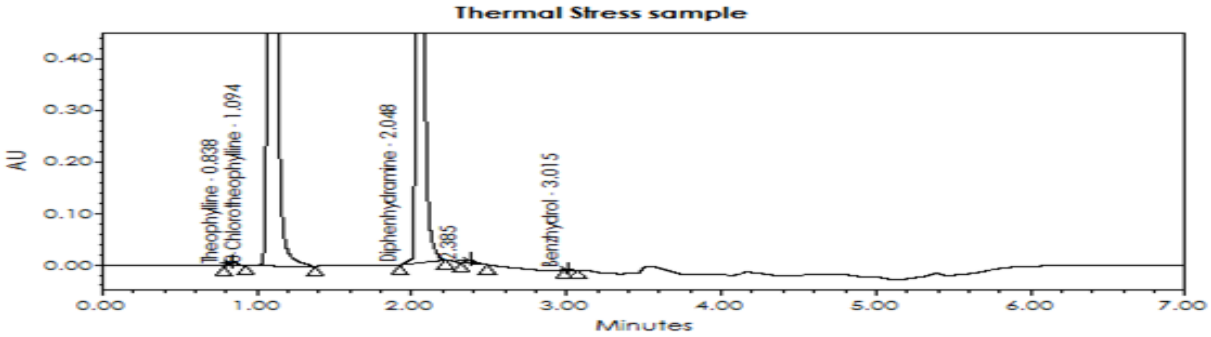

Figure 5: Chromatograms for the degradation conditions

Table 1: Critical process parameters for the screening DOE

Factor	Lower level	Higher level
Concentration of TEA (% v/v)	0.5	2.0
Acetonitrile in Mobile phase A (% v/v)	25	35
Methanol in Mobile phase B (% v/v)	81	90

Table 2: Two level factorial design for the critical process parameters

Factor	Lower level	Higher level
Gradient time program (Minutes)	1.8	2.6
Acetonitrile in Mobile phase A (% v/v)	28	35
Methanol in Mobile phase B (% v/v)	85	95

Table 3: Central composite design factorial design runs

Run	% Acetonitrile in Mobile phase A	% Methanol in Mobile phase B	Gradient time for % Mobile phase A
1	33	99	93
2	35	91	93
3	35	99	93
4	33	91	93
5	34	88	95
6	35	91	97
7	34	95	95
8	34	95	95
9	34	95	95
10	34	95	95
11	34	100	95
12	34	91	97
13	36	95	95
14	34	95	95
15	34	95	98
16	33	99	97
17	32	95	95
18	34	95	92
19	35	99	97
20	34	95	95

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Table 4: The ANOVA for the RT of DPH (Partial sum of squares-Type III)

Source	Sum of squares	df (degrees of freedom)	Mean Square	F Value	p-value Prob > F
Model	0.58	2	0.29	34.05	0.0012
% Acetonitrile in MP-A	0.47	1	0.47	55.40	0.0007
Gradient time	0.11	1	0.11	12.69	0.0162
Residual	0.043	5	8.536E-003	-	
Cor Total	0.62	7	-		

Table 5: The ANOVA for the R_s between DPH and impurity F (Partial sum of squares-Type III)

Source	Sum of squares	df (degrees of freedom)	Mean Square	F Value	p-value Prob > F
Model	3.64	1	3.64	2187.00	<0.0001
% Acetonitrile in MP-A	3.64	1	3.64	2187.00	<0.0001
Residual	0.010	6	1.667E-003	-	
Cor Total	3.65	7	-		

Table 6: The ANOVA for the RT of Benzhydrol (Partial sum of squares-Type III)

Source	Sum of squares	df (degrees of freedom)	Mean Square	F Value	p-value Prob > F
Model	0.57	2	0.29	135.30	<0.0001
% Acetonitrile in MP-A	0.032	1	0.032	15.02	0.0117
Gradient time	0.54	1	0.54	255.57	<0.0001
Residual	0.011	5	2.122E-003	-	
Cor Total	0.58	7	-		

Table 7: The ANOVA for the RT of Benzophenone (Partial sum of squares-Type III)

Source	Sum of squares	df (degrees of freedom)	Mean Square	F Value	p-value Prob > F
Model	0.70	3	0.23	662.22	<0.0001
% Acetonitrile in MP-A	0.019	1	0.019	53.71	0.0018
% Methanol in MP-B	0.021	1	0.021	58.81	0.0016
Gradient time	0.66	1	0.66	1874.16	<0.0001
Residual	1.401E-003	4	3.504E-004	-	
Cor Total	0.70	7	-		

Table 8: The statistical date of central composite design for R_s between Impurity F and DPH

Std. Dev.	0.083	R-Squared	0.8718
Mean	1.11	Adj R-Squared	0.7564
C.V. %	7.52	Pred R-Squared	0.0148
PRESS	0.53	Adeq Precision	8.366
F-value	7.56	p-value	0.0020

Table 9: The statistical date of central composite design for R_s between Benzhydrol and Benzophenone.

Std. Dev.	0.22	R-Squared	0.8581
Mean	3.88	Adj R-Squared	0.7305

C.V. %	5.69	Pred R-Squared	0.3860
PRESS	2.11	Adeq Precision	7.927
F-value	6.72	p-value	0.0032

Table 10: Summary of method validation for DPH, CT and impurities

Parameter	DPH	CT	Theophylline	Impurity F	Benzhydrol	Benzophenone
LOD (%)	0.01	0.01	0.02	0.01	0.02	0.02
LOQ (%)	0.02	0.02	0.04	0.03	0.04	0.04
% RSD *	-	-	2.0	0.9	0.5	6.4
% Rec ± SD	100.5±3.5	90.6±3.4	100.0±6.1	99.8±3.8	95.1±2.5	96.9±4.6
Correlation(r)	0.999	1.000	0.999	0.999	0.995	0.999
R2 value	0.999	0.999	0.999	0.998	0.990	0.998

*Repeatability

CONCLUSION

In the present work, development of a sensitive, rapid, stability indicating and QbD compliant method for the estimation of impurities of Dimenhydrinate in its pharmaceutical formulation was achieved. The method is flexible enough to allow routine laboratory variations. The CPP's responsible for separation of critical band pairs have been identified and studied in detail. The design space has been established. The statistical assumptions have been verified and confirmed through experimentations. The CQA's of method were identified by scientific judgment and were optimized considering the routine laboratory variations during actual usage of method. The method is ecofriendly with very less consumption of solvents, chemicals, instrument power and waste production. With a runtime of 7 minutes, the throughput of method increased by more than 5 times compared the existing methods. Furthermore, the newly developed method was validated as per ICH method validation guidelines. It is proved that the method is suitable for the intended purpose.

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