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METHOD DEVELOPMENT AND VALIDATION OF BUCLIZINE HYDROCHLORIDE ASSAY AND ITS ORGANIC IMPURITIES IN API AND PHARMACEUTICAL FORMULATION BY USING ORTHOGONAL DETECTOR TECHNIQUES (UHPLC-MS) WITH DESIGN OF EXPERIMENTS

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ABSTRACT

To develop and validate a sensitive and stability indicating gradient compatible design of experiment (DOE) based reverse phase ultra-high performance (UHPLC-PDA) liquid chromatography with photodiode array (PDA) and mass spectroscopy (MS) of buclizine hydrochloride (BH) assay and organic impurities (OI) in active pharmaceutical ingredient (API) and pharmaceutical formulation (PF). The chromatographic conditions were optimized using a Zorbax SB-C18 analytical UHPLC column with the dimensions (100 x 2.1) mm and 1.8 µm particle sizes. The mobile phase consisted of 35 mM ammonium formate pH 4.5 (solution A) and 0.02% formic acid in methanol (solution B) with gradient elution as mentioned time (min) % Solution B: 0/70, 0.5/50, 2/80, 6/95, 8/95, 8.2/70 and 10/70.The flow rate was at the rate of 0.4 ml/min and the detection wavelengths were 230nm. The column was maintained at 45°C and the injection volume was 2 µL. Stability of BH sample in different conditions was investigated by exposing the drug to stress study utilizing acid, base, oxidation, thermal, humidity and photolytic. The method was developed in two phases, screening and optimization. During the screening phase, the most suitable stationary phase,

organic modifier, and pH were identified. The optimization was done for secondary influential parameters—column temperature, gradient time, and flow rate using fourteen experiments—to examine multifactorial effects of system suitability parameters and generated design space representing the robust region. A verification experiment was performed within the working design space and the model was considered to be accurate. There was no interference from excipients, impurities or degradation products at the retention time of BH for 4.0 min indicating the specificity of the method. The drug showed good stability under base, oxidation, thermal, humidity and photolytic conditions, but significant degradation was observed under acidic conditions**.** The procedure was validated for specificity, linearity, accuracy, precision and robustness. The degradation products were well resolved from BH and its impurities. The obtained LOD (Limit of detection) values are 0.004% to 0.01% and LOQ (Limit of quantification) values are 0.01% to 0.03% of impurities. A sensitive, rapid, specific and stability indicating gradient reverse phase UHPLC-PDA - PDA with MS (Orthogonal detectors) method for the determination of BH for the assay and OI was successfully developed with DOE. The present method is able to quantify BH and all potential impurities in a shorter (10 min) run time. DOE, statistically based experimental designs proved to be an important approach in optimizing selectivity-controlling parameters for the assay and OI determination in BH API and PF.The developed method was validated to be specific, linear, accurate, precise and robust. The peak purity and LC-MS test results confirmed that the BH peak was homogenous in all stress samples and the mass balance was found to be more than 99%, thus proving the stability indicating power of the method

KEYWORDS: UHPLC-PDA and MS, Orthogonal Detectors, TGA, DOE (Design of Experiments), BH, Stress studies.

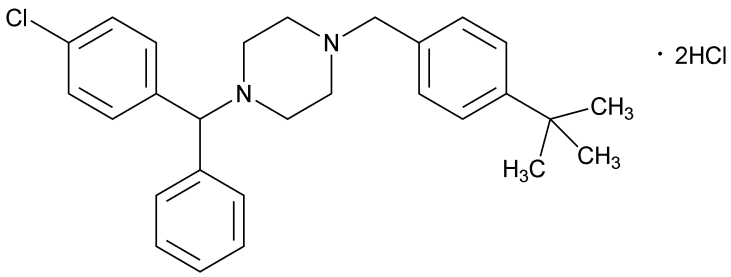
INTRODUCTION

Buclizine Hydrochloride (BH) is an antihistaminic agent or an antiemetic drug of piperazine derivative family used to prevent or treat nausea, vomiting and dizziness resulted from motion sickness. The mechanism by which BH exerts its antiemetic and antimotion sickness effects is not precisely known but may be related to its central anticholinergic actions. It is also utilized in the management of allergic conditions (such as pruritic skin disorders). Chemical structures of BH and its organic impurities showed Figure. 1. The aim of this method is to focus on the Design Space (DS) of the analytical method. The DS requirement of the ICH Q8 states that the DS is a region where process parameters ‘‘have been shown to

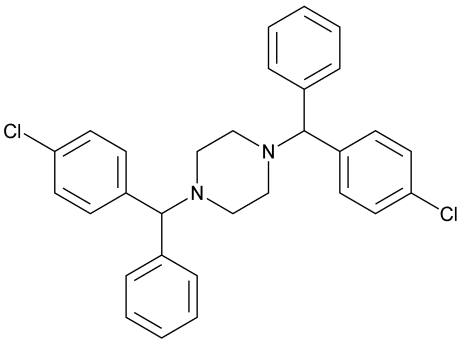
provide assurance of quality''. An additional opportunity for the DS for analytical procedures is the possibility to move inside the DS without the need to initiate a regulatory post-approval change process.

DOE is a systematic method to determine the relationship between factors affecting a process and the output of that process. In other words, it is utilized to find cause-and-effect relationships. This information is needed in order to manage process inputs in order to optimize the output. The developed method, experimental conditions were factorial and deliberately changed. The experimental design approach can be helpful to optimize the separation and to help out in the development of better understanding of the interaction of several chromatographic factors on separation quality. The goal is the assessment of critical factors that effect on the critical quality attributes (CQA) like in retention time, retention time ratio, USP resolution and tailing of peak, etc. next step is creation of experimental design and mathematical model that expresses the relation between the factors and response. In this work, important chromatographic factors were selected based on preliminary experiments and prior knowledge and optimized by a central composite design (CCD) experiment. The DOE of the assay and OI method was checked by injecting five injections of robustness solution and evaluated retention time, retention time ratio, resolution between BH & its impurities, tailing factor and % RSD of BH peak.

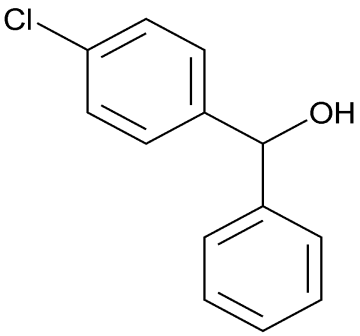
Literature reveals that there are only few analytical methods for the estimation of BH in bulk and pharmaceutical dosage forms HPLC (4)[4to7] and Spectrophotometric (2).[8to9] This method has a long run time as well as does not describe the design space or interaction study of independent factors as per recent FDA guidelines.[13] Regulatory authorities (FDA, ICH, etc.) are promoting and requesting the application of experimental design approach to understand chromatographic selectivity and support better method control, including method transfer. They include UV, HPLC methods but none has reported the indicating UHPLC method for the quantification of process related impurities and degradants of BH. However, the separation and determination of potential process-related impurities in the bulk and a finished product of BH has not been addressed. The present work has been aimed to apply systemic method development to build in a more scientific and multifactorial way for the development of a new rapid UHPLC method for the separation of BH and its impurities in the API and PF using a stable band column.



BH



Impurity-1



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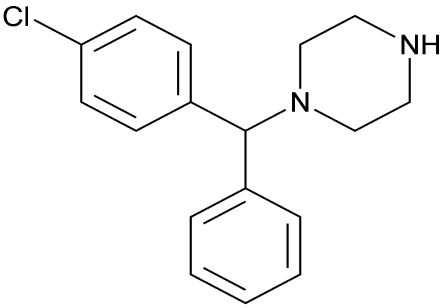
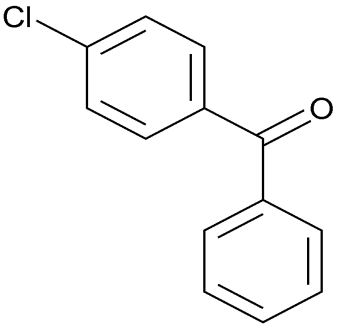


Impurity-3



Impurity-4

Fig. 1: Chemical structure of BH and its impurities.

MATERIALS AND METHODS

Chemicals and reagents

Samples of BH API, tablets and its related impurities were available from USP-India, Hyderabad, India and water from sartorius, resistance 18.2 (MegaΩ-cm), ammonium formate, methanol, hydrochloric acid and sodium hydroxide (Merck grade).

All other organic solvents used were either of analytical reagent or the high-performance liquid chromatography grade.

Instrumentation

The UHPLC-PDA system consisted of a Waters H-Class system with Empower 3 software. Agilent –MS 6460 Triple Quad with LC-1290, Balances: Sartorius, Photo stability chamber (SUNTEST XLS+), Hot Air Oven (Memmert), Humidity Chamber (ESPEC).

Chromatographic conditions

Compounds were separated on Zorbax SB-C18, 100 × 2.1 mm, 1.8 µm column with mobile phase containing 35mM ammonium formate pH 4.5 and 0.02% formic acid in methanol with gradient composition. The flow rate of the mobile phase was 0.4 mL min-1 with a column temperature of 45°C and detection wavelength at 230nm. Injection volume of 2µL. The final gradient elution was optimized as follows: (Tmin/ %B) 0/70, 0.5/50, 2/80, 6/95, 8/95, 8.2/70 and 10/70.

Compounds were detected on an Agilent – LCMS 6460 Triple Quad. The typical operating source conditions for MS scan of BH and its impurities in positive ESI-AJS mode were optimized as follows : Gas temp: 70°C, Gas flow: 10 Lit min-1, Nebulizer: 45 Psi, Sheath gas temp: 400°C, Sheath gas flow: 11 Lit min-1and capillary voltage: 2000 V.

Software

Design of Experiments (central composite), Response Surface function and data analysis calculations was performed by using Design-Expert_ version 10.0.3.1.

Sample and solution preparations

Diluent: A mixture of water and methanol (5:95 v/v) was used as a diluent throughout the experiment for dissolution of the samples.

Preparation of BH assay standard and sample solution

A stock solution of BH (1000 µg/mL) was established by dissolving an appropriate amount in the diluent. Working solution of BH at the concentration of 150 µg/mL was prepared from the above stock solution for assay determination in the diluent.

Preparation of assay sample for BH tablets

Twenty tablets were weighed and average weight of the tablet was calculated and finely powder tablets. Tablet powder equivalent to 15 mg of BH was transferred in to a 100 mL volumetric flask. To this added 70 mL of diluent and sonicated for 30 minutes with intermediate shaking. The solution was subsequently diluted to 100 mL with the diluent and

centrifuged at 3000 rpm for 10 min. The supernatant clear solution (150 µg/mL) was collected, filtered through 0.22 µm filter and used as the sample solution.

Preparation of organic impurities (OI) standard preparation

A stock solution of BH and its impurities (20 µg /mL) was established by dissolving an appropriate amount in the diluent. Working solution of organic impurities of 1 µg/ mL was obtained from the above stock solution in the diluent.

Preparation of BH organic impurities sample preparation

Taken BH about 10000 µg to a 10 mL volumetric flask and dissolved and diluted to volume with diluent (1000 µg/mL).

Preparation of BH and spiking with organic impurities preparation

Taken BH about 10000 µg to a 10 mL volumetric flask and added 0.5 mL of impurities stock solution (20 µg/ mL) dissolved and diluted to volume with diluent (API 1000 µg/ mL and impurities with 1 µg/mL).

Preparation of BH tablet OI sample preparation

Twenty tablets were weighed and average weight of tablet was calculated and finely powder tablets. Tablet powder equivalent to 100mg of was transferred in to a 100mL volumetric flask. To this added 70 mL of the diluent and sonicated for 30 minutes with intermediate shaking. The solution was subsequently diluted to 100 mL with diluent and centrifuged at 3000 rpm for 10 min. The supernatant (1000 µg/mL) was collected, filtered through 0.22 µ filter and used as the sample solution to determine OI.

Preparation of Robustness solution

Taken BH about 15000 µg to a 100 mL volumetric flask and added impurities stock solution (20 µg/ mL) dissolved and diluted to volume with diluent (API 150 µg/mL and impurities with 1 µg/mL).

Chromatographic purity by UHPLC for Potency evaluation

Around 5mg of sample was taken to a 50 mL volumetric flask and dissolved and diluted to volume with the diluent and injected in UHPLC (100 µg/mL).

Weight Loss (%) from TGA for Potency evaluation

Around 5 to 10mg of the sample was taken and analyzed with 5°C linear ramp up to suitable temperature of the respective compounds using TGA (TA-Q50 Model) instrument.

Relative retention factor evaluation

About five solutions containing mixture of API and impurities was prepared with respect to BH limit 0.10% (i.e. 0.10% is considered as 100%, Then 50%, 100%, 150%, 200% and 300%). The prepared solutions were injected into the UHPLC system.

Method Validation

The method was validated with respect to system suitability, specificity, precision, sensitivity, linearity, accuracy and robustness.[10-12]

System suitability

System suitability parameters were performed to verify the system performance. System precision was established on six replicate injections of OI standard preparation and five replicate injections assay standard preparation. All the important chromatographic characteristics, including the relative standard deviation, peak tailing, theoretical plate number and resolution were measured. These all system suitability parameters covered the system, method and column performance.

Specificity

Stress studies were made with an initial concentration of 200 µg/mL of BH in active pharmaceutical ingredients (API) and formulated sample to provide the stability-indicating property and specificity of the method. Intentional degradation was attempted by the stress conditions of exposed to acid (0.1N HCl for 1 hour reflux at 80°C), base (0.1 N NaOH for 24 hour at room temperature), oxidation (6% peroxide for 24 hours at room temperature), Thermal (Exposed at 105°C for 48 h), humidity (Exposed to85°C and 85% RH for 3 days) and photolytic stress (1.2 million lux hours followed by 200 watt-hours per square meter) and Ultrasonic for 1hr.

Precision

The precision of the assay organic impurities method was checked by injecting six individual preparations of BH and BH Tablets (1000 µg/mL) spiked with each 0.1% of impurity-1, impurity-2, impurity-3 and impurity-4. Intermediate precision (ruggedness) was evaluated by

a different analyst in the same laboratory. Assay method precision was evaluated by performing six independent assays of a sample solution of BH and BH Tablets against a qualified standard and calculation of the RSD (%). Intermediate precision of the assay method was evaluated by a different analyst in the same laboratory.

Limit of detection (LOD) and limit of quantification (LOQ):

A signal to noise ratio 3:1 is generally considered acceptable for estimating the detection limit. A typical signal to noise ratio is 10:1is generally considered acceptable for estimating the quantification limit.

Linearity and Range

Impurity linearity solutions were prepared from stock solutions at five concentration levels from 0.05 to 0.3% of analyte concentration. The peak area versus concentration data was subjected to least-squares linear regression analysis. The calibration curve was obtained by plotting impurity areas against the concentration is expressed in µg/ mL. Assay linearity solutions were prepared from stock solution at five concentration levels from 50% to 150% of analyte concentration. The peak area versus concentration data was subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting BH areas against the concentration expressed in µg/ mL.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the observed value. Impurity accuracy of the method was proved at three different concentration levels. The analysis was carried out by spiking all the impurities on the API and formulation samples at 0.05, 0.10, 0.15 and 0.30% of BH concentration (1000 µg/ mL). The percentage means recoveries at each level for all the impurities were calculated. Assay accuracy of the method was demonstrated at three different concentration levels. The analysis was carried out assay on the API and formulation samples at 50, 100 and 150% of BH concentration (150 µg/ mL). The percentage mean recoveries at each level for BH calculated.

Stability in solution and in the mobile phase

BH spiked samples (Impurities spiked at 0.1% of analyte concentration) and BH assay samples were prepared in the diluent and leaving the test solutions at room temperature. The spiked sample and assay samples were injected at 0, 24, 48 hrs time intervals. The peak area

was estimated, and the consistency in the % variation each impurity at each interval was checked. The prepared mobile phase was retained constant during the study period. The mobile phase study was demonstrated by injecting the freshly prepared sample solution at distinct time intervals (0-2 days).

RESULTS AND DISCUSSION

Method development and optimization

BH and its impurities are polar and medium-polar compounds. Analytical method development is more preferable reverse phase. Initially, we tried different reverse phase columns like C8, phenyl, cyano, and C18 for separation of BH and its impurities. But phenyl, cyano column showed poor separation and broader peak shape because of high silonol interactions. In C8 separation is satisfactory but broader peak shape.

Therefore, we considered only C18 column for an optimization study. The overlay UV spectra of BH and its impurities, which indicates that 230nm is the optimum wavelength to detect BH and its impurities with good response as well as minimum base line noise. The mobile phase pH is an important factor that drives the selectivity of the method due to differences in the pKa of molecules. Preliminary method development was tried on three different pH 2.5, 4.5 and 6.8 based on reference.[4] However, high tailing (>2), no proper peak shape was observed at 2.5 and 6.8. We observed at pH4.5 peak shape and resolution are appropriate. But baseline drift was not appropriate. We have tried with 10mM to 50 mM strengths of ammonium formate buffer, observed at 35 mM baseline is appropriate. Mainly organic modifiers for reversed-phase include acetonitrile, methanol and in some cases, n-butanol and tetrahydrofuran. Due to the extreme UV cut off as well as presence of peroxide impurities in tetrahydrofuran that affect the stability of analytes, hence n-butanol and tetrahydrofuran was avoided for selection of organic modifier. We have used methanol because of its cost, good solubility in all buffers and it acts as lewis acid by donating hydrogen that improves the peak shape of BH and added formic acid to methanol (Solution-B) for the improvement of the baseline. Finally we have observed excellent baseline, better peak shapes and separation within 10 minutes. Best results were achieved with 35mM Ammonium formate buffer, pH-4.5 adjusted with formic acid. The mobile phase-A consists of buffer and mobile phase-B consists of 0.02% of formic acid in methanol. The optimized flow rate was 0.4 mL. min-1, column oven temperature was set at 45°C and the elution was carried out with upto 10 min. The UHPLC-PDA gradient program was set as: time (min)/%

solution B: 0/70, 0.5/70, 2/80,6/95, 8/95, 8.2/70 and 10/70. The typical chromatogram where the separation was achieved is illustrated in fig. 2 and fig. 3 Zorbax SB- C18 is a reversed-phase packing that can be employed to basic, neutral or acidic samples. The exhaustive makes it ideal for use with basic compounds, especially those that produce poor peak shapes on other columns. These columns can be used for a wide range of applications over a pH range of 1–8, accommodating most popular mobile phases. In the present study 0.10% specification was kept for the four process impurities with respect to 1.0 mg/mL. Finally, DS was generated to avoid the post approval changes in any parameter that may lead to pay a high cost for any of the company. Operation within the DS will result in a product that complies with the defined quality. Shows fig. 4 work flows of a systematic development approach for LC method development. No formulation excipient interference observed from tablets.

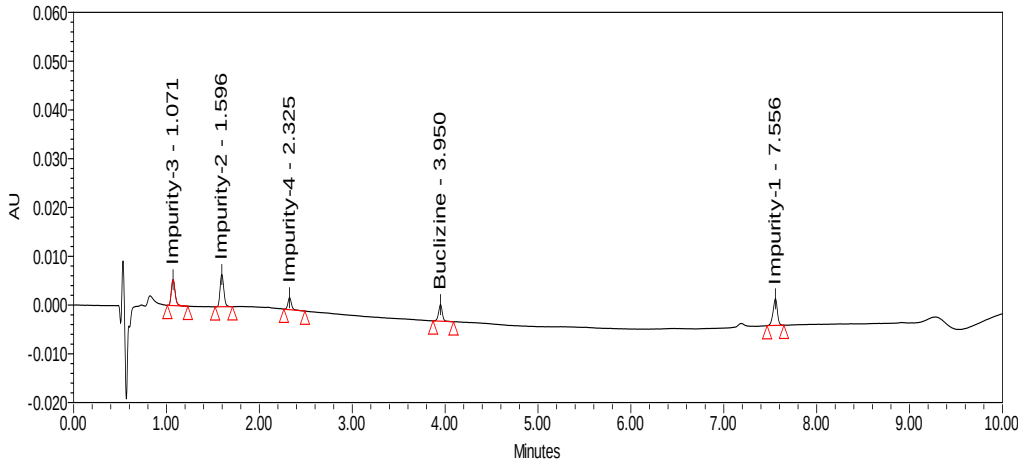


Fig. 2: Typical chromatogram of organic impurities standard.

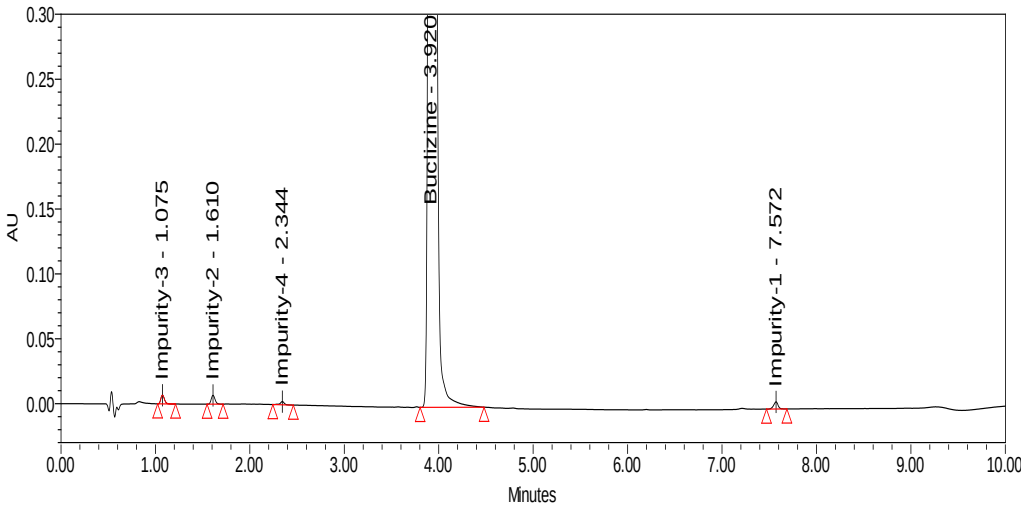


Fig. 3: Typical chromatogram of spiked organic impurities standard.

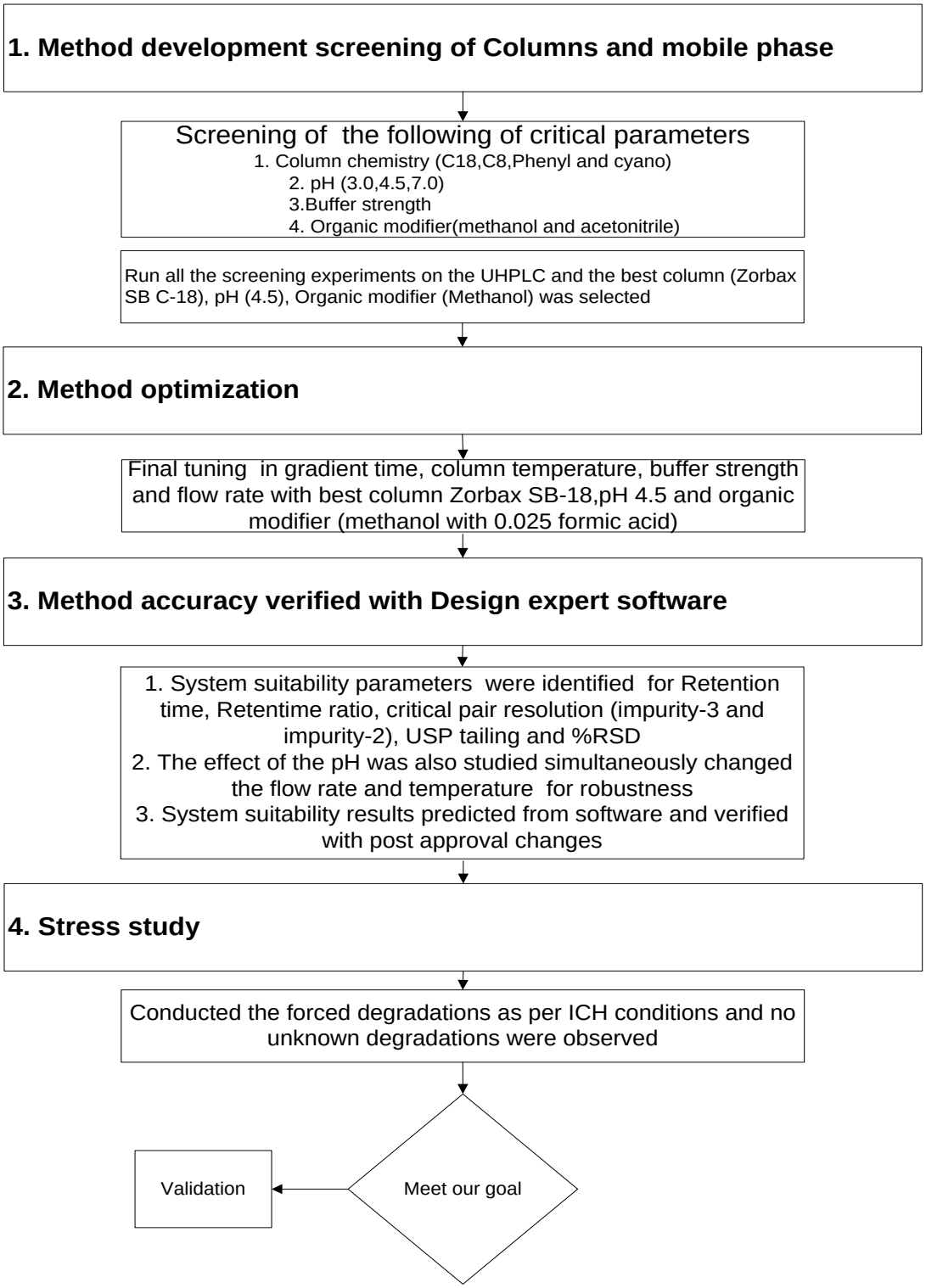


Fig. 4: Work flows of a systematic development approach.

DS generation with DOE and verification of model

To study was conducted with 14 different experiments shown in table 1 after processing all the optimization experiments using empower software, all the system suitability results were transferred into the Design Expert modeling Software.

Generally, the retention time of the BH peak in the chromatogram for identification of analysis. The effect of these parameters were studied simultaneously and made few observations from the contour plot shown in Fig.5 *as design space*. Based on the color code, the working region can easily be identified. Retention time maps represent the value of the critical BH RT with warm "red"colors showing large values (4.4), "Yellow" colors showing desired values (4.0 to 4.2) and "blue "colors, les values (3.8). More DS showing the map was 4.0 to 4.2 as yellow regions with effect of flow, pH variation and software predicted value is 3.78.

The retention time ratio of BH of peak in the chromatogram for accurate quantitative analysis. The effect of these parameters were studied simultaneously and made few observations from the contour plot. Based on the color code, the working region can easily be identified. Retention time ratio of BH and close eluting peak of BH.

Maps represent the value of the "blue "color region shows less values (0.58). More DS showing in the map is 0.6 to 0.62 in the region of "Greenish yellow and greenish blue regions with effect of flow and pH variation and software predicted RT ratio value is 0.60.

The USP Tailing of BH of peak in the chromatogram of assay analysis. The effect of these parameters were studied simultaneously and made few observations from the contour plot. Based on the color code, the working region can easily be identified. USP Tailing maps represent the value of the critical BH USP tailing with warm "blue and light blue "colors showing values (1.16 to 1.12), "Green"color showing desired values (1.22 to 1.24) and more DS showing in the map is 1.26 to 1.28 as yellowish green regions with effect of flow and pH variation and software predicted RT ratio value is 1.27.

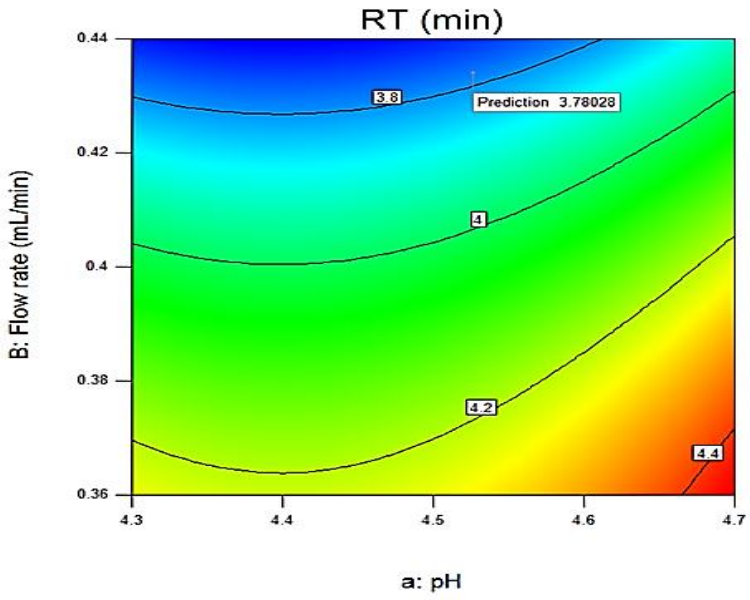
The resolution should not be less than 2 for any of the peak pairs in the chromatogram for accurate quantitative analysis. Therefore, the lowest resolution peak pair (impurity-3 and impurity-2) was considered as critical peak pair and its resolution is the key separation interest of the method. Based on the color code, the working region can easily be identified. Resolution maps represent the value of the critical resolution, with warm bluish region showing less resolution value (6.8), bluish green colors showing resolution values (6.9) and green color region shoes highest resolution values (7.0). The effect of flow and pH variation and software predicted resolution value is 6.90.

The effect of these parameters were studied simultaneously and made on the separation of critical pair.

The system suitability results verified between Software predicted and original method and the accuracy of the results found more than 90% showed in table 2.

Table 1: Design of Experiments method conditions.

Design of Experiments				
Experiment	**Buffer**	**pH**	**Flow rate (mL/min)**	**Temperature (°C)**
Original method	35 mM Ammonium formate	4.5	0.4	45
DOE-1	35 mM Ammonium formate	4.3	0.36	40
DOE-2	35 mM Ammonium formate	4.3	0.44	40
DOE-3	35 mM Ammonium formate	4.3	0.36	50
DOE-4	35 mM Ammonium formate	4.3	0.44	50
DOE-5	35 mM Ammonium formate	4.7	0.36	40
DOE-6	35 mM Ammonium formate	4.7	0.44	40
DOE-7	35 mM Ammonium formate	4.7	0.36	50
DOE-8	35 mM Ammonium formate	4.7	0.44	50
DOE-9	35 mM Ammonium formate	4.5	0.36	45
DOE-10	35 mM Ammonium formate	4.5	0.44	45
DOE-11	35 mM Ammonium formate	4.5	0.4	40
DOE-12	35 mM Ammonium formate	4.5	0.4	50
DOE-13	35 mM Ammonium formate	4.3	0.4	45
DOE-14	35 mM Ammonium formate	4.7	0.4	45



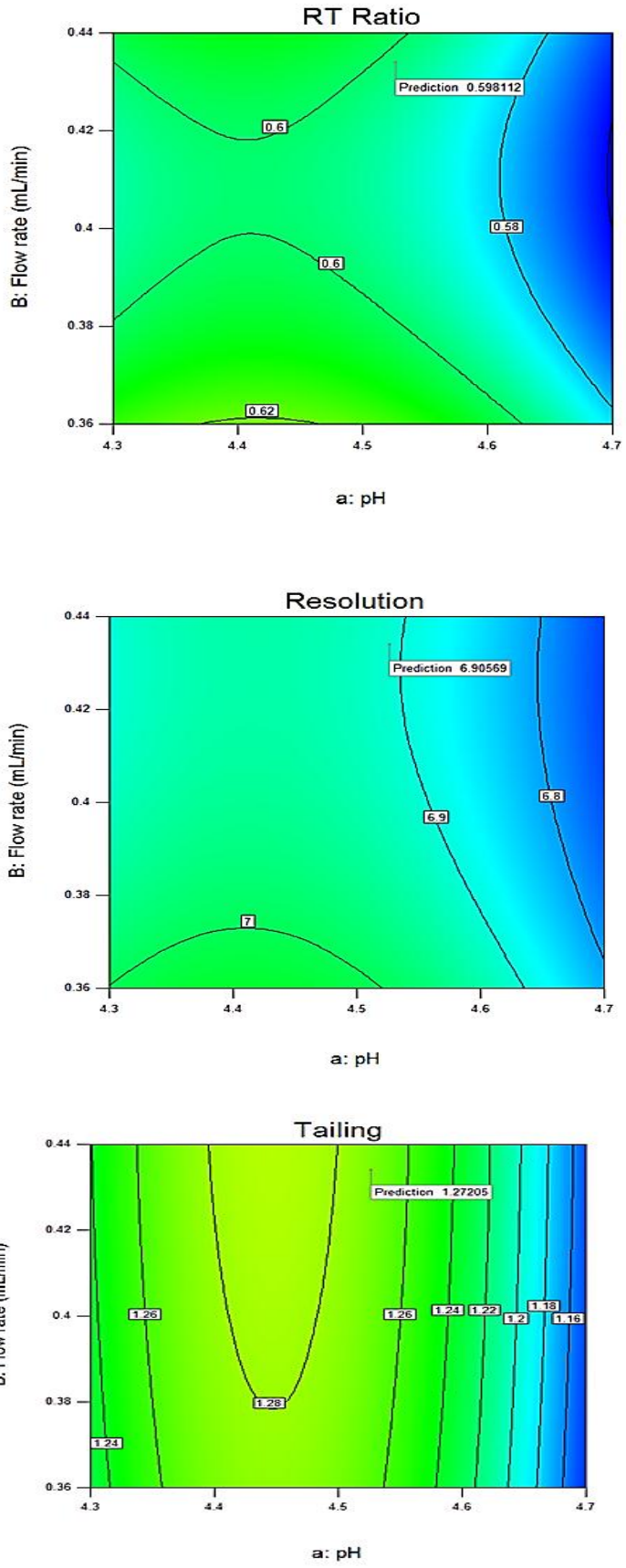


Fig. 5: Design space for Buclizine Retention time, Retention time ratio, USP Tailing and Resolution pH_Flow rate model.

Table 2: Design space for Buclizine Retention time, Retention time ratio, USP Tailing and Resolution pH_Flow rate model.

Verification studies for system suitability				
S.No	**System suitability**	**Design Expert software predicted**	**Original method reported**	**% Accuracy**
1	Retention time (min) (Buclizine)	3.78	3.91	96.68
2	Retention time ratio (Impurity-4 and Buclizine)	0.60	0.63	95.24
3	USP Resolution (Impurity-3 and Impurity-2)	6.91	7.27	95.05
4	USP Tailing (Buclizine)	1.27	1.37	92.70

Specificity – Stress study

All stress samples were analyzed with the aforementioned UHPLC conditions using a PDA detector and MS to monitor the homogeneity and purity of the BH peak and its organic impurities. Individual impurities, tablet samples and BH sample were verified and proved to be non-interfering with each other thus proving the specificity of the method. There is no interference at the RT (Retention Time) of BH and all organic impurities from the other excipients. The drug showed significant degradation under acidic stress conditions Fig.6 and Fig.7. The drug showed to be stable under base, peroxide, thermal, photolytic, humidity and ultrasonic stress studies. It is interesting to note that all the peaks due to degradation were well resolved from the peaks of BH and its impurities. The purity threshold was found higher than angle, suggesting that the peak is pure. The degraded sample solutions of BH were assayed against the qualified standard for mass balance study. The mass balances of stressed samples were found to be more than 99%. Thus, the method is considered to be "Stability-indicating". The specificity results and acid chromatogram along with peak purity and m/z values were shown *Table.5.* Homogeneity of all degradation samples are confirmed by molecular mass.

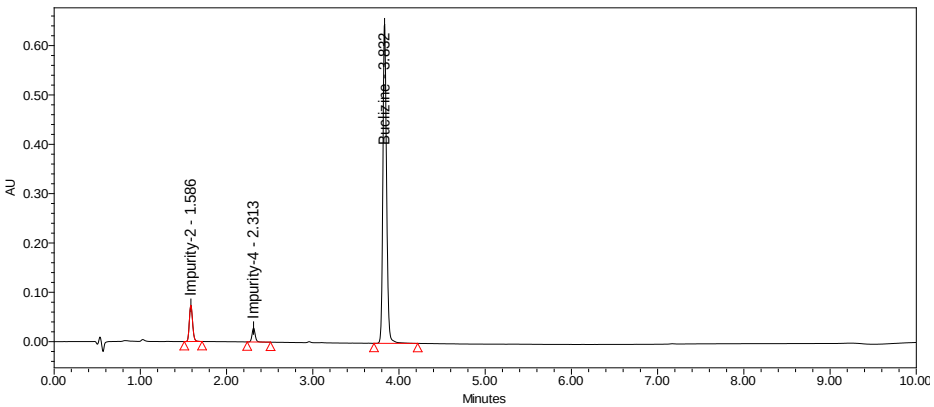


Fig. 6: Typical chromatogram of Acid stressed sample.

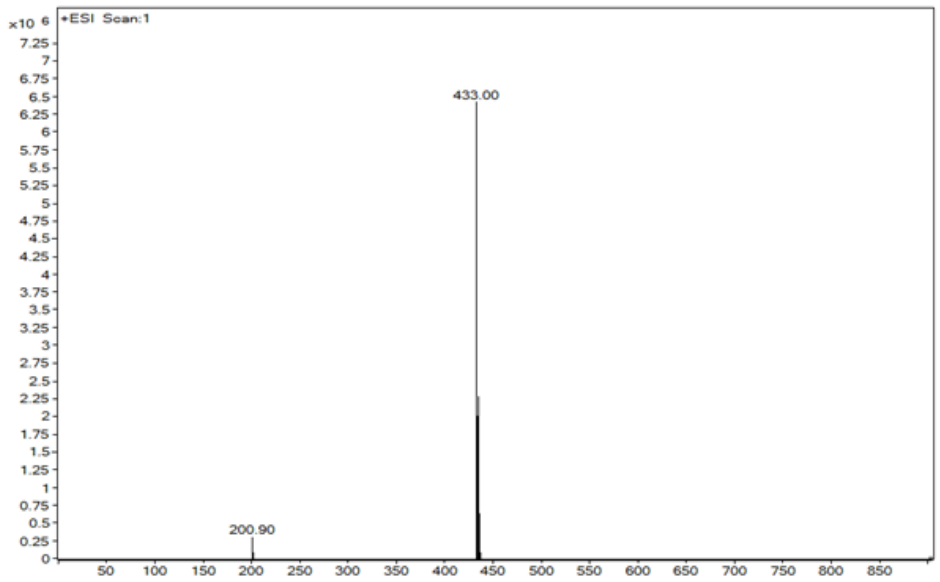


Fig. 7: Mass spectra for Acid degraded sample (Buclizine).

Table 3: Forced degradation data for Buclizine HCl.

Degradation conditions	Buclizine HCl				
	% degraded	Purity angle	Purity Threshold	Mass balance (%)	m/z value of Buclizine
0.1N HCl Refluxed 1hr at 80°C	14.4 Impurity-2: 8.9 Impurity-4: 5.5	0.176	0.277	99.3	433.0
0.1N NaOH bench to at 24 hrs at Room temperature	No degradation	0.118	0.273	99.8	433.00
Stressed with 6% H2O2 24 hours kept on bench top at Room temperature	0.35	0.213	0.273	99.6	433.00
Thermal at 105° C for 48 hrs	No degradation	0.161	0.278	99.8	433.00
Exposed to Visible light for about 1.2 Million Lux-hours and UV light for about 200 Watt-hours / meter square	No degradation	0.176	0.279	99.7	433.00
Humidity 85% RH and 85° C for 3 days	No degradation	0.243	0.277	99.6	433.00
Ultrasonic for 1 hr	No degradation	0.183	0.273	99.9	433.00

The developed method was validated as per ICH guideline (Q2 R1).

System suitability

Results from system suitability study were given in table 4.

Table 4: System suitability

System suitability from Organic impurities solution				
Compound name	**RT***	**RRT#**	**USP Resolution**	**% RSD**
Impurity-3	1.07	0.27	-	0.40
Impurity-2	1.60	0.40	7.07	0.60
Impurity-4	2.33	0.59	10.16	0.32
Buclizine	3.95	1.00	22.79	0.17
Impurity-1	7.56	1.91	45.16	0.23
System suitability from assay standard				
Compound name	RT*	USP Tailing	USP Plate count	% RSD
Buclizine	3.88	1.10	46465	0.09
Buclizine Tablet Assay				
Compound name	RT*	USP Tailing	USP Plate count	
Buclizine	3.89	1.10	42094	

Precision and intermediate precision

The RSD (%) of BH peak during the study of assay precision was below 0.5%. The RSD (%) of the peak area for impurity-1, impurity-2, impurity-3 and impurity-4 in the study of organic impurity method precision was within 2.0%. These results confirm the precision of the analytical method is good. The RSD (%) of the assay results achieved in the study of intermediate precision was within 0.5% and RSD (%) for impurity-1, impurity-2, impurity-3 and impurity-4 were well within 2.0%, confirming the ruggedness of the method.

Limit of detection and limit of quantification

Limits of detection for impurity-1, impurity-2, impurity-3 and impurity-4 was 0.005, 0.004, 0.010 and 0.005% of the analyte concentration (i.e. 1000 µg mL^{-1}), respectively, for a 2 µL injections. The limits of quantification for impurity-1, impurity-2, impurity-3 and impurity-4 were 0.01, 0.03, 0.01 and 0.01% of the analyte concentration (i.e. 1000 µg mL^{-1}), respectively, for a 2 µL injections. The precision (as RSD) at the LOQ concentration was below 5% for all impurity-1, impurity-2, impurity-3 and impurity-4.

The typical chromatogram of LOQ solution is shown in Fig.8.

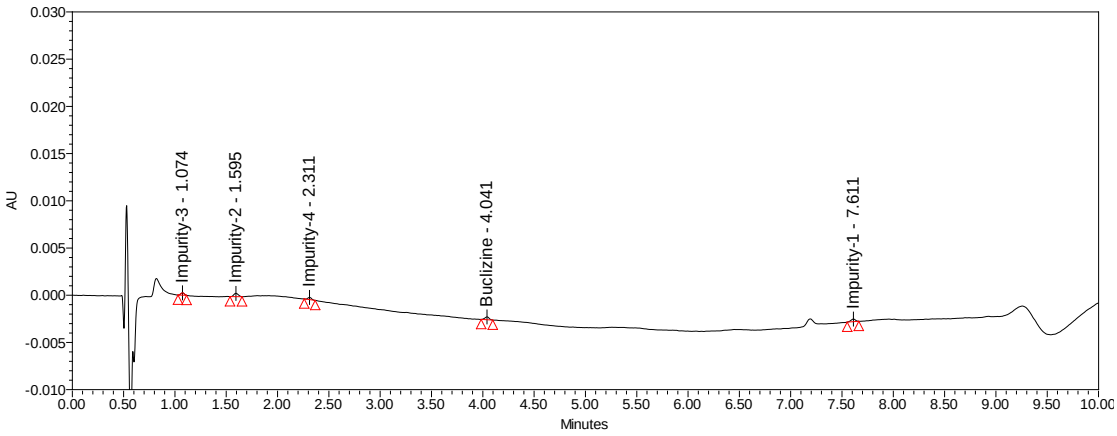


Fig. 8: Typical chromatogram of LOQ solution.

Linearity

A linear calibration plot was obtained for the assay method over the calibration range tested, i.e. 75–225 μg mL^{-1}. The regression coefficient (R^2) was 0.99998, the slope 12046, and the *Y*-intercept 9204.8x. A linear calibration plot was obtained for the OI method over the calibration range tested, i.e. from the 0.05 to 0.3% for impurity-1, impurity-2, impurity-3 and impurity-4. The regression coefficient was 0.999 for all impurities. This result is indicative of the excellent correlation between the peak area and concentrations of impurity-1, impurity-2, impurity-3 and impurity-8 and impurity-4.

Accuracy

Recovery (%) of BH ranged from 100.2 to 100.7% for drug samples and from 99.6 to 100.9% for pharmaceutical dosage forms. Recovery of Impurity-1, impurity-2, impurity-3 and impurity-4 from API samples ranged from 98.9 to 101.5% and 98.9 to 101.3% of dosage sample.

Solution stability

No significant changes were observed in the content of the BH method during solution stability and mobile phase stability experiments when performed. Solution stability and mobile phase stability experimental data confirms that the sample solution and mobile phases used during the assay and impurity determination were stable for at least 48 h at room temperature.

Potency and Relative retention factor evaluation

Potency evaluation

The potencies of all the impurities and API was found to be more than 98.0%. The obtained potency values were tabulated in table 6 and thermogram is shown in Fig.9. The proposed UHPLC method was aimed at developing chromatographic system capable of eluting and resolving BH, its three process known impurities and its degradants. Photo diode array detector is a universal detector which is most preferred for UHPLC analysis in UV-Visible region. Potency evaluation for API and impurities are essential, as it gives the exact content of the compound. Generally potency evaluation for pharmaceutical impurities is carried out by performing organic tests like UHPLC, GC and inorganic tests like LOD, ROI etc. Sometimes due to lack of sample quantity in sufficient amount and its high cost such analysis becomes difficult, for such samples thermo gravimetric analysis (TGA) technique is the best choice for potency evaluation. This technique is very useful to evaluate potency with 5 to 10mg of sample with less run time. This technique involves loss of weight against temperature. The only limitation is impurities must be thermally stable. In the present study of BH and its impurities are thermally stable.

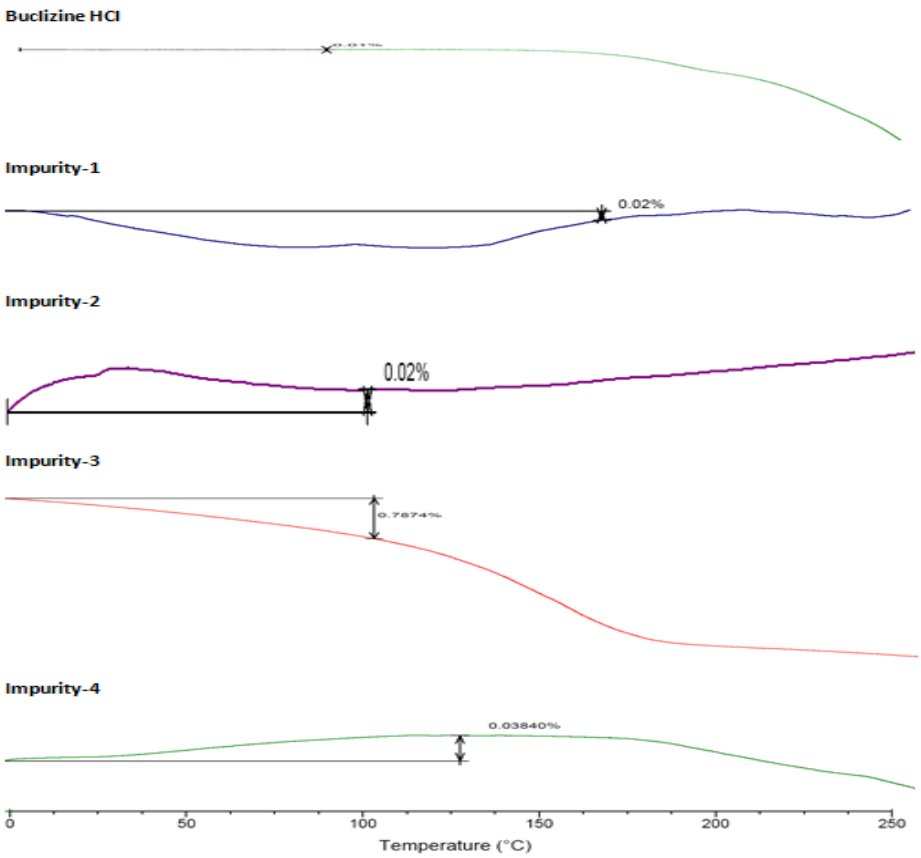


Fig. 9: Thermo gravimetric thermo grams BH and its Impurities.

Relative retention factor evaluation

For these five solutions containing mixture of API and impurities, graph of concentration versus response for impurity and standard solutions was plotted. The slope of individual linearity plots and the correlation coefficient obtained for impurities and standards was more than 0.999. RRF values are tabulated in table 5. Relative Response Factor (RRF) is an analytical parameter used in chromatographic procedures to control impurities/ degradants in drug substance and drug product. RRF is utilized to correct the difference in detector response of impurities with analyte peak. As per United States Pharmacopoeia (USP), relative response factor is the ratio of the responses of equal amounts of the impurities and the drug substance.

Table 5: Potency and RRF results with TGA and UHPLC.

Potency and RRF results					
S.No	**Name**	**% of weight Loss from TGA**	**Purity by UHPLC (%)**	**Potency**	**RRF values**
1	Buclizine Hcl	0.01	99.85	99.84	1.00
2	Impurity-1	0.02	99.34	99.32	1.92
3	Impurity-2	0.02	99.75	99.73	1.98
4	Impurity-3	0.79	99.10	98.32	1.64
5	Impurity-4	0.04	99.98	99.94	0.70

DISCUSSION

Previously reported methods addressed only quantification of BH alone with longer gradients by using regular columns (250mm, 5_m), while the present method is able to quantify BH and all potential impurities in a shorter (10 min) run time. Preliminary method development was tried on reference.[4] However, high tailing (>2), no proper peak shape was observed. Moreover, the extensive literature survey revealed that there is no RP-HPLC method available for a single method of BH API and PF using an experimental design approach (Quality by Design). A few analytical methods have been declared in the literature for the determination of BH alone. They include spectrophotometric methods for BH in pharmaceutical dosage form. This method has a run time of more than 10 min as well as does not describe the design space or interaction study of independent factors as per recent FDA guidelines.[13]

Proposed UHPLC-MS method has been developed for BH and its impurities using systematic approach, with the aid of modeling software Design Expert@. This procedure was rapid and sensitive compare to other reference procedures[4,5,6-9] performed the specificity, linearity, accuracy, precision, LOD & LOQ and robustness of the method to cover the validation range

from 0.05% to 0.15% of impurities and 80% to 120% of the assay. The obtained LOD values are 0.001% to 0.015% and LOQ values are 0.003% to 0.05% of impurities. Recovery data state that the proposed method is accurate and reproducible. The observed linearity correlation was >0.99. This indicates that the method was linear. The lower value of the % RSD of the assay and its impurities indicates that the method is precise. These results concluded that the proposed method is speedy, sensitive and specific method for the analysis of BH API and PF.

CONCLUSION

A novel simple and sensitive reverse phase stability indicating UHPLC-MS method has been developed for BH API and PF and its impurities using a systematic approach, with the aim of modeling software Design Expert@ with orthogonal detectors techniques. Previously reported methods addressed only quantification of BH alone with longer gradients by using regular columns (250mm, 5_m), while the present method is able to quantify BH and all potential impurities in a shorter (10 min) run time. The present study will aid the manufacturers and suppliers of BH to quantify and qualify the purity based on degradation data. The UHPLC-MS method is validated as per ICH guidelines and found to be more specific, precise, linear, accurate, rugged, and most robust. Hence, this method would be suitable for process development and quality assurance of BH in the bulk drug as well as formulation.

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AUTHORS CONTRIBUTION

J. Satish as the first author had done almost all the work of this publication. P. Padmanabharaju helped as a second analyst to execute the studies. M. Ajay Babu helped for the processing and collecting the data. K. Eswara Raju has done a review of data and manuscript. P. Radha Krishnanand was guided for the conceptualization of the study and writing the publication.

CONFLICT OF INTERESTS

All the authors declare that they do not have any conflict of interest.

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