Synthesis based related substances Separation, Isolation, and characterization by LCMS in seizures control entity

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Abstract- Carbamazepine is top essential listed medicine under anticonvulsant, it is alone or with combination with others medication used to control seizures chronic or acute. It is mainly working by reducing abnormal electrical activity towards the brain. But the recent complaint this medicine is, it containing several number of impurities in different monograph books and as per the route of synthesis, different number of reactant impurities which is not listed in pharmacopoeias. The present work aim to develop novel separation condition for the simultaneous estimation of listed impurities as well as unknown impurities. The RP HPLC method was chosen to develop ideal separation. C_{18}packed (250 cm×4.6 cm, i.d. 5 µm particles) used as a stationary phase, acetonitrile and buffer (50mM ammonium formate) at the ratio of 60: 40 v/v was used as a mobile phase. The detection was achieved at 275nm. Drug substance and all the associated impurities well resolved each other and there was no interference obtained from the diluent. The developed method validated as per ICH Q2 (R1) guidelines. In addition four new impurities were separated. Those new impurities were structurally characterized as per LCMS data. To conclude, the developed method can successfully adopt for the simultaneous estimation of carcinogenic reactant based impurities. This study provided approach knowledge to upgrade the list of impurities in the monograph books.

Index Terms- Carbamazepine, RP-HPLC, LC-MS, Acetonitrile, Impurities (bromobenzene, nitro toluene, iminostilbene, iminodibenzyl).

I. INTRODUCTION

Carbamazepine (CBZ) is off white or almost white crystalline powder poorly water-soluble substance, classified under class II drug as per Biopharmaceutics Classification System. It has great intestinal penetrability. CBZ is available in four polymorphic forms [1]. Out of four, the dehydrated form has a very limited absorption and dissolution rate [2]. Different polymorphs form various in the air crystal structures, melting points, chemical reactivity, solubility and compatibility with various additives when converting into formulations. Hence its bioavailability also gets influenced by different forms [3]. CBZ molecular formula is $C_{15}H_{12}N_2O$, chemically known as 5H-dibenzazepine-5-carboxamide, possess a molecular weight of 236.27g/mol. It is an iminostilbene derivative with a tricyclic structure. The reported melting point range was 189 – 193°C, pKa was 7.0 and 2.45 is partition coefficient (log P) value. The CBZ lead was discovered in 1953atBasel, Switzerland. The scientist Schindler and F. Hafliger synthesized the drug and it was marketed for the treatment of trigeminal neuralgia [4]. Later in the year 1962 CBZ molecule was identified and repurposed as a potential compound for epilepsy [5]. From the year 1986 [6]. CBZ is used as a tonic for chronic seizures, partial seizures, and prime widespread seizures from the childhood. It is also used for other neurological disorders like bipolar disorder. The detailed literature review explored that, CBZ was qualitatively and quantitatively estimated using many techniques. CBZ stability-indicating methods have been developed by using a UV-visible spectrophotometer [7], CBZ was estimated in bulk drug and tablet dosage form. [8]RP-HPLC-based method developed for the determination of CBZ in pharmaceutical preparations. Simultaneous HPLC methods have been reported for the estimation of Levetiracetam and CBZ [9]. CBZ, phenytoin, and 10, 11-carbamazepine epoxide were estimated simultaneously in human plasma by HPLC [10]. Phenytoin and CBZ estimated in Serum by RP-HPLC [11]. Cross-reactivity nature of CBZ with Carbamazepine 10, 11-epoxide and its assay was performed [12], CBZ and its impurities like Iminostilbene (IMSB) and Iminodibenzyl (IMDB) estimated in solid dosage [13] form the drug estimated in Parenteral Nano emulsions performed by HPLC as a tool [14]. CBZ *in-vitro* release study was performed by HPTLC [15], therapeutic drug monitoring of CBZ, lamotrigine and valproic acid were performed by using LC-MS/MS [16] in dried blood spot. Oxcarbazepine and CBZ genotoxic affect assisted in Drosophila [17]. The thorough literature review revealed that ample number of study performed in the estimation, few studies were reported related to CBZ impurities related biosafety. The statement clearly exploded that the synthesis related impurities qualitative and quantitative estimation were not detailed among the field.

The first major problem associated with CBZ is, the official monographs prescribed many number of impurities, and those are not same. They are10, 11- di-hydro carbamazepine (Impurity A), 9-methylacridine (Impurity B), N-carbonyl carbamazepine (Impurity C), Iminostilbene (Impurity D), Iminodibenzyl(Impurity E), 5-cholrocarbonyliminostilbene(Impurity F), , 10-bromocarbamazepine(impurity G) [18]. The drug and prescribed impurities structures were shown in figure 1 (here). Secondly the CBZ is synthesized based on many pathways, through literature reviled that CBZ had been synthesized based on thirty-six routes [19]. Which consist of many reactants, but based on the compilation of all the routes, four impurities have been shortlisted for this work based on their individual CAS (Chemical Abstracts Service) toxicity reports [20]. These impurities are observed from the three specific synthetic routes which involves impurities D, E, H and I which is consider as carcinogenic substances as per CAS. Toxicity [20]. The short listed four synthetic routes shown in figure 2-4. However the different pharmacopeia like, Indian Pharmacopeia [21] denote two impurities(i.e impurity A and E),British Pharmacopeia prescribed with seven numbers(i.e impurities A, B, C, D, E, F, G), the US Pharmacopoeia covers six number of impurities(i.e

impurities A, B, C, E, F, G) but Japanese pharmacopeia didn't describe any impurities. Their simultaneous estimation method have not been published Elsevier. In this context, the present aim to develop a qualitative and quantitative simultaneous separation method for iminostilbene (IMSB), Iminodibenzyl (IMDB), Nitro toluene (NT), Bromobenzene (BB) impurities in the presence of CBZ. These impurities have been identified as carcinogenic substances when they are explored to the human body for long time during chronic disease management. In additionally the current work also aimed to develop the force degradation study profile to assess the unknown impurities which was not previously mentioned by the official books. (Figure 1-5)

II. MATERIALS AND METHODS

Gifts sample of Carbamazepine bulk drug procured from Intus Pharmaceutical Private Limited, Pune and the impurities IMSB (Imp-D), IMDB(Imp-E) NT(Imp-H) and BB(Imp-I)are purchased from Sigma Aldrich. D ionized water was obtained from Milli-Q plus water purification system. HPLC grade Methanol and acetonitrile, dichloromethane and methanol bought from Merck India Limited. Analytical grade hydrochloric acid, Sulfuric Acid and hydrogen peroxide (30%) formic acid and analytic reagents ammonium formate was bought from Qualigens India Limited. The UV-Spectroscopy model is UV-1700Pharmaspec, Shimadzu and the software is UV-Pro 2.31. Shimadzu prominence SPD 20A module equipped with a 2487 UV detector using a C18 column (250 cm×4.6 cm, i.d. 5 µm particles) the software is Lab Solution. The instrument LCMS was Shimadzu 2020 and the software is Lab Solution.

2.1 Preparations of standard stock solutions

10 mg of standard CBZ was weighed accurately and transferred to 10 ml volumetric flasks dissolved with few drops of acetonitrile. Further the solution diluted up to 10µg/ml solution using acetonitrile. These solutions stored under refrigerator conditions until the analysis get over.

2.2 Preparation of impurities stock solution

The stock solution was prepared by 10 mg of standard impurities (IMDB and IMSB) transferred to a volumetric flask and make up 10 ml with acetonitrile and prepare 1mg/ml stock solution. And for BB and NT impurities by dichloromethane and methanol respectively.

2.3 Preparation of impurities working standards

IMSB and IMDB standard impurity 1µg/ml solutions were prepared from stock solutions using acetonitrile as a solvent. NT impurity was prepared by dissolving 1ml in 10 ml of methanol, final concentrations diluted up to 5ng/ml with acetonitrile form the above stock solution. 1ml of BB working standard solution diluted up to 10ml with Dichloromethane and further diluted up to 100ng/ml with acetonitrile

2.4 Preparation of 0.05M ammonium formate buffer

3.153gm of ammonium formate was dissolved in 1000 ml of milli pore water. The solution pH was adjusted to 3.1±0.1 by using formic acid.

2.5 Preparation of mobile Phase

The mobile phase was prepared by mixing acetonitrile and buffer at the ratio of 60:40% V/V. the mobile phase sonicated to remove dissolved gases.

2.6 Preparations of linearity solutions

To evaluate the method response related varying concentrations the linearity and range had been demonstrated. The serial dilutions were prepared for IMDB, IMSB, NT and BB. For IMDB and IMSB the concentrations range were 0.075-0.2µg/ml. NT concentration was 0.1875-0.5ng/ml and BB was diluted to 3.75-10ng/ml. The linearity response was verified by linearity equation method. The linearity response have been plotted for serious of concentrations against the peak area. The slope equations and r2 value have been calculated. The linear response were statistically evaluated to analyse the significance, the obtained liner response were shown in Figure 6(here).

2.7 Optimization of detection wavelength

To find the optimistic common isosbestic point for the detections of all the impurities, all the diluted impurities have been scanned to record UV spectrum, the resulted overlay spectrum mentioned in Figure 2 (here). The λmax were found to be 282nm, 260.50nm, 258nm, and 260nm, 247nm for CBZ, IMDB, IMSB, NT and BB respectively. It is showing in the figure 7 (here).

2.8 Optimization of separation conditions

Shimadzu prominence SPD 20A module equipped with a 2487 UV detector was selected to develop separation. C_{18}packed (250 cm×4.6 cm, i.d. 5 µm particles) stationary phase was chosen as stationary phase and acetonitrile and buffer (0.05M ammonium formate) at the ratio of 60: 40 v/v was used as a mobile phase. The injection volume was 20µL.flowrate was 1ml/min with gradient condition. The temperature of the column was maintained at room temperature. By adjusting different parameters like mobile phase ratio, organic phase ratio and flow rate the separation was optimised. In the stabilised condition impurities and drugs have been injected and the chromatograms were recorded. To evaluate the performance of developed method, method had examined for all the method and chromatographic related evaluation parameters and their results were statistically evaluated. The retention time for the CBZ and IMSB, IMDB, NT and BB were5.450 min, 14.308 min, 21.008 min, 26.025 min, and 41.025 min respectively the obtained chromatogram were shown in Figure 8 (here).The pH related optimization provided well separated and symmetric peak. During the production of the system to boost the theatrical plates, reduce the tailing effect. Formic acid was used to boost the pH of the ammonium formate buffer to 3.1. For acetonitrile, the mobile phase composition was set at 60:40percent v/v and, respectively, buffer. The chromatographic condition of RP-HPLC was described by mobile phase, stationary phase, solvent ratio, detection limit, flow rate, Injection volume, Retention time, Elution mode and run time. Mobile phase used Acetonitrile: buffer at 60:40%V/V as an isocratic condition. The column was used Shim pack (150x4.5mm.,i.d. 5µ) as a stationary phase. The wavelength was selected 275 nm at flow rate 1ml/min. the injection volume was 20µg/ml for 45min run time. (Figure 8 here)

2.9 Stress degradation study protocol

For small molecules, the forced degradation analysis is considered a critical analytical element of the drug development program. Forced degradation, usually referred to as stress testing,

and is performed to demonstrate the precision of using high-performance liquid chromatography to establish a stability-indicating analytical process, i.e. a single analytical method capable of distinguishing the degrading peaks from the peak of the drug substance/drug product. Stability studies need to be conducted to propose the shelf life of new drug substances and/or drug products, as per the International Conference on Harmonization (ICH) guidelines (Q1A). Studies of shelf life are part of numerous FDA regulatory submissions. To suggest the shelf life of a drug substance and/or drug product, three types of stability studies typically need to be conducted: rapid stability (ACC), intermediate stability (INS), and stability of regulated room temperature (CRT). The length of the accelerated analysis is approximately six months and the intermediate stability and controlled stability experiments at room temperature take approximately 12 to 24 months. To determine the intrinsic stability of the molecule, a stability analysis is performed and the drug substance/drug product is required to degrade/decompose and produce other molecules known as impurities during the study. Various stress conditions are purposely applied during forced degradation studies to degrade/decompose the main compound and produce impurities that should be isolated from the main compound and each other. Forced degradation studies are also a tool for estimating the degrading/decomposed impurities that occur during stability studies and are used to suggest new drug substances and/or drug products for shelf life.

Initially 100 µg/ml CBZ were involved for all the stress degradation. 1 N Hydrochloric acid, 1 N Sodium Hydroxide were exposed to drug for 48 hours at 25ºC. Oxidative experiments performed for 72 hours at room temperature with 30% hydrogen peroxide solution. Neutral hydrolysis performed in water medium and 1M Hcl, photo-degradation by exploring the solutions to sunlight for 2 days during the daytime. Necessary controls were preserved in the dark. Additionally, at 50 ^{0} C for 4 days and 60^0 C for 7 days, the drug powder was exposed to dry heat to assess the drug substances change when it exposed to heat. Samples were withdrawn at a reasonable time and subjected to LCMS analysis by following sufficient dilution.

Acid treated sample were analysed for 0,2,4,8,12,24,48 hours intervals. Likewise base degradation base samples analysed additionally at 48 hours. Peroxide based stress sample analysed for 1 week. Photolytic degradation samples examined after 2 days exposure to direct sunlight.

2.10 Method validation

The developed RP HPLC method for Carbamazepine quantification was validated according to the ICH Guidelines for specificity, linearity, precision and accuracy, detection limit and quantification limits, robustness and ruggedness. The analytical method developed in this study was validated using the International Conference on Harmonisation's guidelines (ICH).

2.10.1 Accuracy& Recovery

Accuracy and recovery parameters are the standard protocol to verify the developed method quantification and recovery capacity. To evaluate accuracy the estimation were performed for 3 times, the average of 3 times results were evaluated for related standard deviation. In addition the recovery was evaluated by using three different concentration levels in triplicate.

2.10.2 Preparation of assay solutions from marketed formulations

Amount equivalent to 10 mg CBZ was transferred to tablet was transferred to a 100 ml volumetric flask. Initially the substances was extracted by sonication in mobile phase and final volume made with mobile phase. Then it filtered through a 0.45 m filter. The final concentration (10 µg / ml) was obtained by diluting with mobile phase. The final solution was injected in to HPLC, average results of triplicate, standard deviation, and percent RSD were measured.

2.10.3 Specificity/ Selectivity

The capacity of a system to calculate the analyses response in the presence of other excipients and possible impurities is referred to as specificity. The specificity was demonstrated by comparing the chromatogram of the medication extracted from the tablet to that of the regular solution for excipients, possible impurities, and other degradants. At the Carbamazepine retention time, there was no disturbance in the sample solution.

2.10.4 Precision

Interday and intraday precision studies were used to determine the method's precision. The process precision was investigated using six separate injections of three different concentrations 0.2, 0.15, 0.075µg/ml for CBZ, IMSB, IMDB and 0.5, 0.375, 0.1875ng/ml for NT, 10, 7.5, 3.75ng/ml (HQC,MQC, and LQC levels). Intraday precision/repeatability was achieved by injecting the above samples on the same day, while interday precision was achieved by injecting the same samples three times. The mean and percent relative standard deviation values were computed. Recovery experiments were used to determine the method's accuracy. It was achieved using the standard addition process, which involves applying a known concentration of a standard drug to the actual sample and analysing it under ideal chromatographic conditions. The percent mean recovery, standard deviation, and percent relative standard deviation were determined for three different stages of recovery.

2.10.5 Linearity and Range

The linearity of an analytical method refers to the response that is linearly proportional to the analyses concentration over a given range. For IMDB and IMSB, the proposed method's linearity was measured over a range of 0.075-0.2 g/ml. The range for BB is 3.75-10ng/ml, and for NT it is 0.1875-0.5ng/ml.

2.10.6 Detection Limit and Quantification Limit

When a system can detect low concentrations of analyse, it is said to be sensitive. The detection limit (LOD) and quantification limit (LOQ) of the established RP HPLC method were determined by analysing low concentrations of the standard solution. The LOD is the analyse concentration at which a measurable response can be obtained. The LOQ is the smallest concentration of analyse that provides an accurately quantifiable response. The following formula can be used to determine the LOD and LOQ values LOD = 3.3/S and LOQ = 10/S, Where σ = Standard deviation of the response; S = Slope of the deviation curve.

2.10.7 System Suitability

System suitability parameters play an important role in the development and validation of analytical methods. They ensure that the system operates at its best. Parameters in chromatography Number of potential plates(N), retention time

(Rt), resolution (Rs), and peak asymmetric factor (A) were calculated for six replicate results.

2.10.8 Robustness and Ruggedness

The methods' ruggedness and robustness were investigated by modifying the experimental conditions (analyst, reagent source, and various brand columns) and optimising the chromatographic conditions (pH, mobile phase composition, and mobile phase ratio and flow rate).

2.10.9 Resolution

The ability of a column to distinguish peaks on a chromatograph is referred to as resolution. The ratio of the distance between two peak maxima to the mean value of the peak width at the base line is known as resolution (R).R = (TB −TA)2 /wA +wB, where TB is component B's retention time, TA is component A's retention time, wA is component A's peak width, and wB is component B's peak width. Components are completely segregated if R is equal to or greater than 1, but if R is less than 1, components overlap.

2.11 Collection of new impurity fractions

In the acid degradation the at 48 hours the drug was getting of its 80% degradation as per ICH guideline and the new impurity/degradant was collected at 6.2 min and 7.1 min in the RP-HPLC at C18 column and the mobile phase ACN and Ammonium formate buffer at 3.1 pH by formic acid as well as the base degradation was also proceed like same acid degradation but the retention time of the new base degradant is 7.4 min and 16.4 min. and the fraction was next send for LCMS system.

2.12 Characterisation of new impurities by LCMSMS

Instrumentation and LC-MS Conditions

The identification were achieved on a Shim-pack (VP-ODS) C18 analytical column (5 mm, 250*4.6mm ID, Shimadzu, Kyoto, Japan) by maintaining the column temperature at 30ºC. A linear gradient elution was used of A (80% acetonitrile) and B (20% ammonium formate buffer solution). The flow rate was 0.5ml/min by solvent splitting. The system operating mode was MRM with positive polarity. The injection volume was 10µl. And the degradant sample runtime was 5 minutes. An Agilent-1100 HPLC system with a binary pump, vacuum degassed, auto sampler, column heater-cooler (Agilent Corporation, MA, USA), and a photodiode array (PDA) detector, coupled with an LC-MSD Trap SL electrospray ion mass spectrometer, was used for tool creation and qualitative analysis. The MS conditions were as follows: 8L/min drying gas (N2), 350°C, 30 psi nebulizer strain, 3.5 kV HV voltage, and a scan range of 50–1000 u. Lab solution software was used to collect the data.

2.13 Preparation of sample for LCMS

The mobile phase was prepared by ACN and buffer solution, the buffer solution was prepared by the ammonium formate and make up the pH 3.3 by adding formic acid. The sample was the degradant product of acid and base by the RP-HPLC method. The mobile phase condition was 80:20 (V/V) ACN: buffer solution. The range was selected for 100-300 molecular weight as the drug CBZ molecular weight is 236.27g/mol. The flowrate of the sample is 0.5ml.

III. RESULTS AND DISCUSSION

Impurity screening has become a requirement of drug regulatory bodies for any new drug applications that are introduced to the market. Transparency in drug efficacy and safety is critical from the consumer's perspective in this regard. The first stage in predicting the known and unknown effects of other chemical substances present in the lead molecule is separation and quantification of those substances from the lead. Analytical method development plays a key role in this process. In present days in present scenario CBZ is the better clam of drug for the treatment of various epilepsy. However, because of presence of reactant based impurities IMDB, IMSB, NT, and BB there is a need for. Despite the fact that several analytical methods were mentioned in the CBZ in the previous research track, there was no clear simultaneous method for separation and quantifying IMDB, IMSB, NT, and BB.

Method development initiated by using several solvent, at the end of method development, acetonitrile and water selected as a, the HPLC method for the drug substance and impurities was used as a starting point. The HPLC column used was a Shim pack 150x4.5mm, i.d. 5µ. Acetonitrile (B) and buffer (ammonium formate) were used in the mobile phase (50:50, v/v) (A). Peaks from the CBZ were observed at 260 nm, which caused significant interference. The sensitivity of the CBZ and impurities (IMDB, IMSB, NT, and BB) was significantly improved when detected at 275 nm, although interference from the CBZ and related impurities, as well as diluent components, was greatly reduced. The optimal separation was achieved by reducing the mobile phase ratio to 60:40 (v/v, B/A).

Validation real goal is to test the developed a method performance under different parameters as per ICH Q2 (R1). The closeness of agreement between the value accepted as a traditional true or accepted reference value and the value found is expressed by the accuracy of an analytical method. To assess precision and recovery experiments were carried out. For CBZ and impurities, the assay were repeated six times, percentage relative standard deviation of the percentage recovery was measured and presented in a table 1 and 2(here). The CBZ in the bulk medication was found to be 99.12 percent w/w according to the data collected. The daily dose of bulk drug contained 25.40, 28.25, 19.2 and 21.40 of IMDB, IMSB, NT, and BB, respectively.(Table 1-2)

The developed analytical method's selectivity was tested by analysing a solution containing CBZ and its associated substances. The CBZ and IMSB, IMDB, NT and BB were eluted at 5.450 min, 14.308 min, 21.008 min, 26.025 min, and 41.025 min respectively. No interferences were found in the chromatogram. The standard chromatogram reported in figure 8(here). The resolution and relative retention time, theoretical plate were expressed in table number 5.

The inter and intraday precession were measured by injecting six individual solutions in the concentration level like 0.3, 0.5, and 0.7 µg/ml. Intra-day variability was measured over one day by the same analyst, while inter-day accuracy was measured over three days by another independent analyst. The standard deviation SD and related standard deviation (RSD) were calculated for the retention time and peak area. The obtained result were within the limit.

The calibration curves for all the analyses under the selected conditions are summarized in Table3 and 4(here). Linear range for IMDB and IMSB were 0.075-0.2µg/ml. NT and BB linearity concentrations were found to be 0.1875-0.5ng/ml for and 3.75-10ng/ml respectively. The slope equation and Correlation coefficients (r2) for impurities was y =370416x + 8841.5, R^2 =

0.9924 was IMDB, For IMSB it was found to be y = 714005x – 14884, R^2 = 0.9901. For NT and BB slope equations were y=38795x-224.14 R^2 = 0.9927 and y = 191137x + 3743.6 R^2 = 0.9916respectively.The linearity analysis was performed under triplicate. The obtained combined linearity curve shown in figure number 6 (here). (Table3-4)

The developed method LOD and LOQ were calculated. The lowest concentration of analyse that produces a detectable response is known as the Limit of Detection (LOD). The Limit of Quantification (LOQ) is the smallest concentration of the analyse that yields a quantifiable answer. At 3 and 10 times the baseline noise, the LOD and LOQ were calculated, respectively. CBZ and IMDB, IMSB, NT, and BB were found was LOD and LOQ of 0.086, 0.071, 0.075, 0.078, 0.732ng/ml and 2.54, 2.621, 2.282, 1.45, 1.32 ng/ml respectively.

Experiments were carried out to test the method's ruggedness and robustness by varying conditions such as column, reagents, instrument, detection wavelength, and data systems. HyperSep™ C18 Cartridges column, apex C18, Phenomenex C18 column have been used to verify the column variation, the analysis were repeated in the Shimadzu HPLC by using the recommended mobile phase system to observe the instrument variations in the chromatogram. There were no significant changes in the chromatogram pattern and retentions times. The actual detection wave length was 275 nm. To check the effect of wavelength in the peak response the wavelength changed to 275±1 nm. The change did not respond majorly in the chromatogram data. The class VP data station and Lab Solution data acquisition software have been used to analyse the HPLC response. The tested parameter responses resulted that the developed method durable and reliable.

The optimised methods system suitability parameters including column efficiency (theoretical plates), capacity factor, Asymmetric factor, resolution, relative retention time and tailing factor calculations were obtained from the chromatogram data to verify the satisfaction. The obtained resolution was 2.575, 5.63, 1.808, and 6.59. The relative retention time for NT, BB, IMSB, IMDB were 0.927, 1.07, 1.029, and 0.98 respectively.

The CBZ stability capability was analysed by stress degradation study protocol. The degradation were performed under degradation conditions like acid, base, hydrogen peroxide and photolytic degradation. The drug responded majorly for the 1M acid and 1M base degradation. After 48 hours of the degradation the degraded solution were subjected to chromatographic separation. In acid degraded solution in two retention time like at 6.2min and 7.1 min two new peaks were observed. Those peaks were not matched with the retention time of standard impurities like IMDB, IMSB, NT and BB. The base degradation responded at 7.4min and 16.4 min. Retention time, those peaks also not matched with the retention time of excited standards. The rate of acid degradation of CBZ was higher than the alkali solution. In the peroxide and photolytic degradation the drug was not responded significantly to identify the new impurities. Hence those retained substance were collected and recovered and involved for LCMS analysis for fragment pattern analysis to predict the unknown structure. The predicted unknown impurities structures mechanism were explained in figure 9, 10 and 11 (here).

IV. CONCLUSION

The method developed in this article for HPLC and LCMS is rapid, responsive, and precise. The method's precision and accuracy are within reasonable limits (ICH Guideline Q2 (R1)). This technique is especially appealing for the quantification of CBZ because of its simplicity and high sensitivity. In addition four new impurities were separated. Those new impurities were structurally characterized as per LCMS data. To conclude, the developed method can successfully adopt for the simultaneous estimation of carcinogenic reactant based impurities. This study provided approach knowledge to upgrade the list of impurities in the monograph books. Routine quality management analysis can also be easily adapted using this approach. A formalised, systematic approach to drug development can make the process more effective. Initial toxicological studies and subsequent comparison of impurity profiles can be used to concentrate a consistency concept on (toxicologically) qualified impurity profiles. As part of this systematic characterization of impurity profiles, LC–MS techniques can be used.

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