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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING AND LCMS COMPATIBLE UPLC METHOD FOR ESTIMATION OF ASSAY, RELATED SUBSTANCES AND DEGRADANTS IN DEXTROMETHORPHAN HYDROBROMIDE ACTIVE PHARMACEUTICAL INGREDIENT.

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ABSTRACT
The current study reports the sensitive and reproducible stability indicating ultra performance liquid chromatography method for quantitative determination of Dextromethorphan Hydrobromide assay and its related substances including process related impurities and degradation products. The drug was subjected to various stress conditions such as hydrolysis, oxidation, photo- and thermal degradations to investigate the stability indicating ability of the method. Significant degradation was observed during oxidative stress. Dextromethorphan Hydrobromide was well resolved from its process related impurities and degradation products formed under stress conditions. Efficient chromatographic separation was achieved on an Acquity; UPLC, BEH; C-18;100 X 2.1mm; 1.7 µm column with the mobile phase consisting of 10mm Ammonium bicarbonate in water and CH3CN/Methanol in a gradient elution mode within a short run time of 13 minutes at a flow rate of 0.3 ml/min. The eluents were monitored by a photodiode array detector at 225 nm and quantitation limits were obtained in the range of 0.1–1.0 µg ml^{-1} for Dextromethorphan Hydrobromide and all its process related impurities. The resolution between DHB and its seven impurities was greater than 2.5 for all pairs of impurities. The developed UPLC method is superior in technology against conventional HPLC with respect to speed, resolution, solvent consumption and cost of analysis. This method is compatible to LCMS analysis which enables to identify the unknown impurities formed in the process. The developed liquid chromatographic UPLC method was validated as per the ICH guidelines.

KEYWORDS: Dextromethorphan Hydrobromide (DHB), UPLC, process related impurities, stability indicating method.

INTRODUCTION
Dextromethorphan Hydrobromide (ent-3-methoxy-17-methylmorphinan) is the dextrorotatory enantiomer of the methyl ether of levorphanol and also methyl analog of dextrorphan that shows high affinity binding nature to the several regions of the brain including the medullary cough center. Dextromethorphan enhances serotonin activity by inhibiting the reuptake of serotonin specific non-opioid binding sites present in the central nervous system which mediate the antitussive effects. DHB is a cough suppressant drug used for pain relief and psychological applications.[1,4]

Impurity profiling of active pharmaceutical ingredients up to their lowest signal to noise ratio or with the minimal specifications for known and unknown impurities is the most challenging task for the method development.[5] The presence of unknown impurities or some known specific impurities at low level may influence the efficacy and safety of the pharmaceutical product.[6] For these reasons limits of impurities at lower level has to be established as per various regulatory authorities.

Ultra Performance Liquid Chromatography (UPLC) system works with advanced technology that brought revolution in liquid chromatography by outperforming conventional HPLC. UPLC shortens the sample run times with improvised resolution and sensitivity. The sub-2-µm hybrid particles showed high mechanical strength which can operate at high pressure. Because if its speed, sensitivity, high resolution and low organic

solvent consumption, this technique has gained considerable attention in recent years in the method development and validations of pharmaceutical and biomedical compounds.[7,9] In the present work this technology has been applied for the method development and validation study for assay and related substances of DHB.

Dextromethorphan Hydrobromide (DHB) is official in Indian Pharmacopoeia[10], United States pharmacopoeia and European pharmacopoeia, its literature study revealed that there are several methods reported for assay analysis and only couple of methods reported for related substances by HPLC. USP Pharmacopoeia method do not have any method reported to separate the impurities.[11] European Pharmacopoeia method showed the separation of the drug along with four impurities (Impurity-A, B, C and D)[12], however it cannot separate all the potential and degradation impurities. There were several HPLC assay methods reported for the determination of DHB either individually or with the combination of other formulated drug products.[13,19] There are also several methods for the determination of Dextromethorphan and its metabolites in plasma and urine by HPLC and LCMS.[20,24] There are couple of methods for determination of related substances in DHB. The RP-HPLC method[25] was reported for simultaneous estimation of six impurities in Dextromethorphan along with Guaifenesin, however this method failed to report and separate Impurity D and also the run time was around 75 minutes. There was one more HPLC method reported[26] for determination of related substances (Six impurities) by HPLC, but this method used non-volatile buffer which is incompatible for LCMS determination to identify unknown impurities and degradants, also this method did not include all the process related impurities of Dextromethorphan Hydrobromide.

According to our extensive literature survey none of the currently available HPLC methods can separate and quantify all the seven process related impurities and degradation impurities of DHB. Further there is no UPLC method reported to determine assay and quantify all the process related impurities in single method. It is therefore felt necessary to develop a stability indicating UPLC method for assay and related substances quantification with shorter run time without compromising on the resolution and sensitivity.

Hence an accurate and reproducible novel UPLC method was developed for quantitative determination of DHB and its disclosed potential seven impurities named Impurity-A Impurity-B, Impurity-C, Impurity-D, N-Oxide Impurity, N-Formyl Morphine and N-Formyl octabase with shorter run time of 13 minutes with improved peak shape and better resolution. This method is LCMS compatible which enables to identify the unknown impurities formed in the process. Chemical structures were shown in Fig-1 and the details of chemical names of all impurities were tabulated in Table-1. This method was successfully validated according to ICH guidelines.[27,29]

MATERIALS AND METHODS

Chemicals and Reagents

High purity ultra-pure water was obtained by using a Millipore Milli-Q water purification system. HPLC grade acetonitrile, methanol were purchased from Merck, Germany. Analytical grade ammonium bicarbonate, HCl, H2O2 (30%w/w) and NaOH were purchased from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India. Dextromethorphan Hydrobromide API, N-Formyl morphine and N-Formyl octabase was obtained from TLC pharmaceutical standards (Ontario, Canada). Impurity-A, Impurity-B, Impurity-C, Impurity-D and Dextromethorphan N-oxide were obtained from Pharm affiliates analytics and synthetics, Haryana.

Instrumentation and Chromatographic Conditions

Ultra performance liquid chromatography

A prominence series Waters Acquity UPLC system equipped with a binary solvent manager pump, an auto sampler and PDA detector with empower-3 software was used for method development, validation and stress degradation studies. The chromatographic column used was Acquity; UPLC BEH -18 column {(100 X2.1) mm; 1.7 µm} from waters. The mobile phase components are (A) 10mm Ammonium bicarbonate in water and (B) Acetonitrile and Methanol in the ratio of 30:70, separation was accomplished in a gradient elution program {time (min)/% B: 0.0/40, 1/40,5/50, 10/80, 13/80,13.1/40} at a flow rate of 0.3 ml/ min. The chromatographic eluents were monitored at a detection wavelength of 225 nm using a photodiode array (PDA) detector. The sample injection volume was 1.5µL.Water and Acetonitrile in the ratio of 30:70(v/v) was used as diluent.

Mass spectrometry

Waters; Ultra performance liquid chromatography coupled to Single Quadrupole (SQD) mass spectrometer equipped with an ESI source was used for identification and characterization of degradation products of Dextromethorphan Hydrobromide. The data acquisition and processing were under the control of Mass Lynx software. The typical operating source conditions for MS scan in positive ESI mode were optimized as follows: the Cone voltage was 25 V; Nitrogen gas was used for nebulization (100 $L.Hr^{-1}$) and drying (1000$L.Hr^{-1}$).

Preparation of stock and working standard solutions

Stock solutions of DHB (10.0 mg mL^{-1}) and all seven impurities (1.0 mg mL^{-1}) each were prepared separately by dissolving the appropriate amounts in the minimum amount of acetonitrile and diluted to volume with diluent. The working standard for related substances analysis was prepared by diluting the above stock solutions in order to obtain 1.0 mg mL^{-1} of DHB mixed with 0.002 mg mL^{-1} of all seven impurities at a level of 0.2%. The working standard solution for DHB assay was

diluted from above stock to obtain 0.25 mg mL^{-1} of DHB.

Specificity and forced degradation

Specificity is the ability of the method to measure the analyte (DHB) response unequivocally in the presence of its possible impurities. The specificity of the developed LC method for DHB was determined in the presence of its process related impurities (Impurity-A Impurity-B, Impurity-C, Impurity-D, Impurity N-Oxide, N-Formyl Morphine and N-Formyl octabase at the level of 0.2%) and degradation impurities. Specificity was shown by separation of DHB and 7 known impurities with resolution factor of greater than 2.5 without interferences of any blank mobile phase peaks. The sample solution include mixture of DHB(1.0 mg mL^{-1}) and 7 impurities(0.002 mg mL^{-1} each) Forced degradation studies can help to identify the likely degradation products, also they in turn can help to establish the degradation pathways and the intrinsic stability of the molecule. DHB(1000 µg mL^{-1}) was subjected to stress conditions such as acidic (1 N HCl, 60°C, 3h), basic (1 N NaOH, 60°C, 3h) and neutral hydrolysis (H2O, 60°C, 3h), and oxidation (3.0% H2O2, 60°c 3h) in solution state. DHB was also subjected to thermal (60°C, 2days) and UV light (254nm) stress in solid state. Different stress conditions were followed to achieve significant degradation. Acid and base hydrolysed samples were neutralized, and all the degradation samples were diluted five times for assay determination. DHB assays were performed by comparison with standard and the mass balances (%assay +%impurities + %DPs) calculated for stressed samples. The degradation samples were injected into an LC-PDA system to check the peak purity and homogeneity of the DHB peak. LC-MS was used for the characterization of the degradation and unknown peaks.

Method Validation

Method validation of the UPLC method was carried out for the determination of related substances (i.e., Impurity-A Impurity-B, Impurity-C, Impurity-D, Impurity N-Oxide, N-Formyl Morphine and N-Formyl octabase) and assay of DHB as per ICH guidelines to demonstrate that the method is appropriate for its intended use.

System suitability

The system suitability test was conducted by injecting six replicates of DHB (1.0 mg mL^{-1}) spiked with 0.2% each of all seven impurities (0.002 mg mL^{-1}) for related substances and injected six replicates of DHB (0.25mg mL^{-1}) for Assay quantification. Resolution between all adjacent peaks, RSD values, tailing factor and theoretical factor were used to confirm system suitability. These solutions were injected throughout the validation studies.

Precision

The system precisions was checked by analysing six replicates of working standard solutions for both assay (DHB - 250 µg mL^{-1}) and related substance (DHB 1000 µg mL^{-1} spiked with 0.2% of each seven impurities (2 µg mL^{-1}) individually. The method precisions for assay and related substances were evaluated by injecting six individual test preparations of DHB (250 µg mL^{-1}) and DHB (1000 µg mL^{-1}) spiked with 0.2% of each seven impurities (2 µg mL^{-1}) respectively. The intermediate precision was evaluated with same concentration solutions used for methods precision prepared separately on a different day by different analysts. The RSD (%) of peak area was calculated for all impurities.

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ values for DHB and related substances (seven impurities) were determined at signal-to-noise ratio of 3:1 and 10:1 respectively by injecting a series of dilute solutions with known concentrations.

Precision at LOQ levels was also determined by injecting six individual preparations of mixtures of all impurities and DHB at their LOQ level. The %RSDs of the areas of each impurity and DHB were calculated for precision studies.

Accuracy

The accuracy of the analytical procedure expresses the closeness between true value and observed value. Accuracy of the related substance method was evaluated by spiking known amounts of the impurities into the test sample (1000 µg mL^{-1}), analysing the same and calculating the percent recovered. For related substances, the recovery studies were performed in triplicate at three concentration levels (50%, 100% and 150%) to specification level (0.2.%) of all impurities (i.e. 1.0, 2.0, and 3.0 µg mL^{-1}) with respective to drug substance concentration 1000 µg mL^{-1}.

The accuracy of the DHB assay method was evaluated in triplicate (n=3) at the three concentration levels 50%, 100% and 150% (i.e., 125, 250 and 375 µg mL^{-1}) of drug substance and the recovery was calculated for each added concentration (spiked).

Linearity

Linearity of the related substance method was established by analyzing series of dilute solutions at six different concentration levels ranging from LOQ to 200% of the specification level (0.2%) of the impurities (i.e. LOQ, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 µg mL^{-1}) spiked into DHB drug substance (1000 µg mL^{-1}). The calibration curves were drawn by plotting the peak areas of impurities against their corresponding concentrations. Similarly, assay method linearity was established by injecting DHB at six different concentration levels ranging from 50% to 200% (i.e. 125, 187.5, 250, 312.5, 375, and 500µg mL^{-1}). The correlation coefficients (r^2), slopes and Y-intercepts of impurities and DHB were determined from their respective calibration plots.

Robustness

The robustness study was carried out to evaluate the influence of small variations in the optimized chromatographic conditions. The factors chosen for this study were flow rate (± 0.03 ml/ min) and Mobile phase composition change (±10.0%). The effect of flow rate was checked with 0.27ml min^{-1} and 0.33ml min^{-1} and the effect of initial mobile phase composition was checked at 36% and 44% of organic solvent mixture. System suitability parameters and changes in assay of DHB were checked. In all the above deliberately altered experimental conditions, the components of the mobile phases were held constant.

Solution stability and mobile phase stability

Solution stability was carried out by storing the impurities standard solution DHB 1000 µg mL^{-1} spiked with 0.2% of each seven impurities (2 µg mL^{-1}) at room temperature for 72 hours. This solution was injected at an interval of 0, 24, 48 and 72 hours. The impurity content and system suitability results were checked at each time interval. The mobile phase stability study was demonstrated by injecting the freshly prepared impurities standard solution at different time intervals (0, 1 and 2 days) keeping the same mobile phase as constant.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Preliminary experiments were carried out to develop a chromatographic system not only capable of eluting and resolving DHB from its process related impurities and stress DPs but also compatible with LC-MS characterization. DHB spiked with 0.2% of each impurity was taken for method development study. To develop an optimal chromatographic method several parameters like column stationary phase, buffer, pH, diluent, detection wavelength for accurate response factor, flow rate and gradient conditions were evaluated to ensure that the method is accurate enough for routine analysis in laboratory.

For the initial stages of method development, Acquity BEH Shield RP-18 column, Acquity; BEH HSS T3 column and Acquity; BEH phenyl column each with 100mm length and 1.7µ particle size were tried using acidic and basic pH buffers. Trifluoroacetic acid was used for acidic conditions and ammonium bicarbonate was used for basic conditions, acetonitrile was used as organic solvent in all the above three columns. A desirable resolution (>2.0) between DHB and impurities was not achieved with several mobile phase compositions and different gradient elution modes. In acidic conditions Impurity-A and N-Oxide impurity were co eluted with DHB in all the three columns. In basic conditions Impurity-D was merged with N-Formyl morphine impurity even though separation of other impurities are reasonably good.

Further trials were conducted using Acquity BEH C-18(100x2.1mm, 1.7µ) column using different mobile phase compositions, initially acidic buffer TFA with acetonitrile solvent was used but the desired separation of impurities with DHB was not found. Literature survey revealed that the pKa of the DHB is around 8.30 and it is known that to achieve good separation and to avoid pH related ionic variation the mobile phase pH should be ± 1.5 of pKa value of the drug. In order to find optimum pH to achieve good separation Ammonium Formate buffer (pH: 5.5) and Ammonium bicarbonate buffer (pH: 7.8) were used using acetonitrile as organic co solvent. In Ammonium Formate buffer desired separation was not achieved Impurity-A and Impurity-D were merged. The Ammonium bicarbonate in water (mobile phase-A) and acetonitrile (mobile phase-B) in gradient elution gave separation of all the impurities but with lack of desirable resolution (>2.0) was not achieved between Impurity-D and DHB. Impurity-D was separated at the tailing end of the DHB peak. So Ammonium bicarbonate buffer with C18 column was fixed, however couple of other parameters were fine-tuned, mobile phase B was changed from acetonitrile to acetonitrile/methanol combination and gradient programmes were modified. Finally, after few attempts by changing acetonitrile/methanol compositions, the best separation was observed in Acquity, BEH C-18 column {(100 X2.1) mm; 1.7 µm}, by using 10mm Ammonium bicarbonate in water (mobile phase-A) and Acetonitrile: Methanol (70:30) v/v (mobile phase-B) with a flow rate of 0.3 $mL.min^{-1}$ with the below mentioned gradient programme shown in T**able-2.** The UV spectrum of DHB and all the seven impurities were correlated and the cross over wavelength of 225nm was fixed for all the validation studies.

The chromatographic separation of DHB (1000µg mL^{-1}) spiked with 0.2% of all impurities (2.0µg mL^{-1}) under optimized conditions is shown in **Fig. 2.**

Method validation

System Suitability

System suitability parameters were evaluated for DHB and its seven impurities, system suitability summarised in Table-3 indicating that the system was suitable for use as the tailing factor for all the peaks were less than 2.0 and the resolution between any of the two adjacently eluting peaks were greater than 2.5. The USP plate count for all the peaks were greater than 5000. These values were fixed as acceptance criteria for all the remaining studies.

Precision

The % RSD in the repeatability of the DHB assay concentration was 0.27% and the % RSD of the peak area for all the seven impurities in related substances concentration was within 1.85%. The % RSD for the method precision repeatability for assay and impurities were 0.16% and 2.31% respectively. The % RSD obtained for the intermediate precision of assay method

was 0.12% and the % RSD for all seven impurities peak areas were within 1.63%. All the values were tabulated and presented in Table-4. These results confirmed that the method was highly precise in terms of repeatability.

Limits of detection (LOD) and quantitation (LOQ)

The determined limit of detection and limit of quantification results of DHB and its related substances were in the range of 0.03-0.3 µg mL^{-1}(LOD) and 0.1-1.0 µg mL^{-1}(LOQ) respectively indicating the higher sensitivity of the method. The %RSD values for precision at LOQ level of peak areas for six injections were below 3.41%. All the detailed values were reported in Table-4.

Accuracy

The method accuracy was evaluated by spiking DHB and its seven impurities at 50,100 and 150% w/w and calculating the % of recovery. The recovery of DHB was in the range of 98.32-102.85% and the recoveries of all the impurities were in the range of 98.0-102.0% as shown in detail in Table-5. The results were consistent with the theoretical values confirming the accuracy of the method.

Linearity

Linear calibration plot for the related substance method was obtained over the calibration ranges tested that is LOQ to 0.4% (LOQ, 50%, 75%, 100%, 125%, 150% and 200%) of the target test concentration. The correlation coefficient results obtained for all impurities were greater than 0.995. The linearity calibration plot for assay method was obtained over calibration range of 50 to 200% and the correlation coefficient value was 0.9995 and the results were shown in Table-5.

Robustness

In all deliberately altered chromatographic conditions (flow rate and mobile phase composition) all analyte's were adequately resolved and elution order remains unchanged. The resolutions between any two adjacent peaks obtained were greater than 2.5 and tailing factors of all peaks obtained were less than 2.0 and the theoretical plates of all the peaks were greater than 5000 indicating the robustness of the method.

Solution stability and mobile phase stability

No significant changes in the content of impurities and no additional peaks observed during solution stability and mobile phase stability carried at specified intervals. The stability study results showed that the sample solutions were stable for 72 hours and the mobile phase was stable for two days without compromising the system suitability results.

Specificity and Force degradation results

The method specificity was confirmed by peak purity studies of DHB and all the seven impurities, the purity angle value of each peak is less than the purity threshold value of each peak indicating the homogeneity of the separated peaks. All the forced degradation samples were analysed with afore mentioned UPLC conditions using a PDA detector to monitor the homogeneity and peak purity of DHB and the other impurities. No considerable degradation of DHB drug substance was observed under acidic hydrolysis, basic hydrolysis, neutral hydrolysis, thermal and UV light stress conditions. Significant degradation of DHB drug substance was observed in peroxide hydrolysis (3%H2O2, 60°C for 3 hours) leading to the formation of around 8.0% of N-Oxide impurity. The chromatograms for the forced degradation were shown in **Fig-3**. The impurity formed in the oxidative conditions was confirmed by co-injection with the system suitability solution. It was also confirmed by LCMS/MS analysis. The LCMS analysis was performed for the degraded solution and the mass of the impurity formed was 287 m/z corresponding to N-oxide impurity. The two unknown impurities formed were not identified by LCMS due to very low level (<0.1%). Peak purity test results obtained from the PDA detector confirmed that the DHB peak was pure and homogeneous in all the analysed stress samples. The mass balance of stress samples was found to be more than 99.0%. The forced degradation results are summarized in **Table 5**. Insignificant change in assay of DHB in the presence of degradant related substances and peak purity results of stress samples confirm the specificity and stability-indicating ability of the developed method.

Table-1: List of impurities with their chemical name.

Compound Name	Chemical Name
DHB	Ent-3-methoxy-17-methylmorphinan
Impurity-A	Ent-3-methoxymorphinan
Impurity-B	Ent-17-methylmorphinan-3-ol
Impurity-C	Ent-3-methoxy-17-methylmorphinan-10-one
Impurity-D	Ent-(14S)-3-methoxy-17-methylmorphinan
N-Oxide Impurity	3-Methoxy-N-methylmorphinan N-oxide
N-Formyl Morphine	3-Methoxy-6,7,8,8a9,10-hexahydro-5H-9,4b(epiminoethano)phenanthrene-11-carbaldehyde
N-Formyl Octabase	(S)-1-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydroisoquinoline-1(1H)-carbaldehyde

Table-2: Gradient Programme.

Time in minutes	% of Mobile phase-A	% of Mobile phase-B
0.0	60	40
1.0	60	40
5.0	50	50
10.0	20	80
13.0	20	80
13.1	60	40

Table-3: System Suitability Results.

Compound	RT(Min)	RRT[a]	RRF[b]	USP Tailing	USP Resolution	USP Plate count
Impurity-B	1.718	0.32	0.91	1.59	-	10885
N-Oxide Impurity	2.947	0.55	0.84	1.06	15.68	18244
Impurity-A	3.527	0.66	0.92	1.79	5.84	17789
DHB	5.312	1.0	1.0	1.55	15.27	27231
Impurity-D	5.725	1.08	1.21	1.93	3.33	37066
Impurity-C	7.601	1.43	0.69	1.16	18.89	166371
N-Formyl Morphine	7.890	1.48	1.25	1.13	3.81	178816
N-Formyl Octabase	9.529	1.79	0.70	1.31	23.57	398470

[a] Relative retention times
[b] Relative response factors

Table-4: Method Validation Results.

Parameter	Impurity-B	N-Oxide Impurity	Impurity-A	DHB	Impurity-D	Impurity-C	N-Formyl Morphine	N-Formyl Octabase
LOD(µg mL^{-1})	0.12	0.03	0.15	0.3	0.15	0.07	0.12	0.15
LOQ(µg mL^{-1})	0.4	0.1	0.5	1.0	0.5	0.22	0.4	0.5
Slope	31130	14688	17303	7099	20203	11486	32694	23410
Intercept	-3397	1899	-468	2230	27.1	566.7	-4474	-3291
Correlation coefficient	0.995	0.995	0.997	0.998[a] 0.9995[b]	0.991	0.995	0.997	0.992
Precision(%RSD)	0.67	0.55	1.85	0.18[a] 0.16[b]	0.97	0.76	0.47	0.51
Intermediate Precision(%RSD)	1.58	0.58	1.3	0.24[a] 0.12[b]	1.01	0.37	0.41	1.63
Precision at LOQ(%RSD)	2.41	2.39	1.42	1.56	1.32	1.61	1.08	3.14
Linearity range(µg mL^{-1})	0.4-4.0	0.1-4.0	0.5-4.0	1.0-1000[a] 125-500[b]	0.5-4.0	0.22-4.0	0.4-4.0	0.5-4.0
% Recovery Amount spiked[c] (%) 50 100 150	 101.0 100.0 99.7	 99.0 101.5 99.7	 101.0 101.0 100.0	 100.7 102.8 98.32	 99.0 99.5 99.3	 102.0 98.0 99.7	 101.0 100.0 100.3	 100.0 100.0 99.7

[a] Values obtained with respect to target concentration of 1000 µg mL^{-1} of DHB
[b] Values obtained with respect to assay concentration of 250 µg mL^{-1} of DHB
[c] Amount of impurities spiked with respect to 0.2% specification level individually to 1.0 mg mL^{-1} of DHB.

Table-5: Summary of forced degradation results.

Parameter	Stress condition	% of purity	% degraded	Purity angle	Purity threshold	% Assay	Mass balance[a]
Acid degradation	1N HCl 60°C; 3 Hrs.	100	ND	0.523	1.853	99.3	99.3
Base degradation	1N NaOH 60°C; 3 Hrs.	100	ND	0.467	0.571	99.5	99.5
Peroxide degradation	3% H2O2 60°C; 3 Hrs.	91.49	8.2	1.692	1.956	92.6	100.8
Thermal degradation	60°C; 2 days	100	ND	0.448	0.530	99.4	99.4
Water hydrolysis	H2O 60°C; 3 Hrs.	100	ND	0.450	0.541	99.3	99.3
Photolytic degradation	UV light; 254nm	100	ND	0.462	0.561	99.5	99.5

ND: Not detected

[a]Mass balance: % assay + % of all degradation impurities

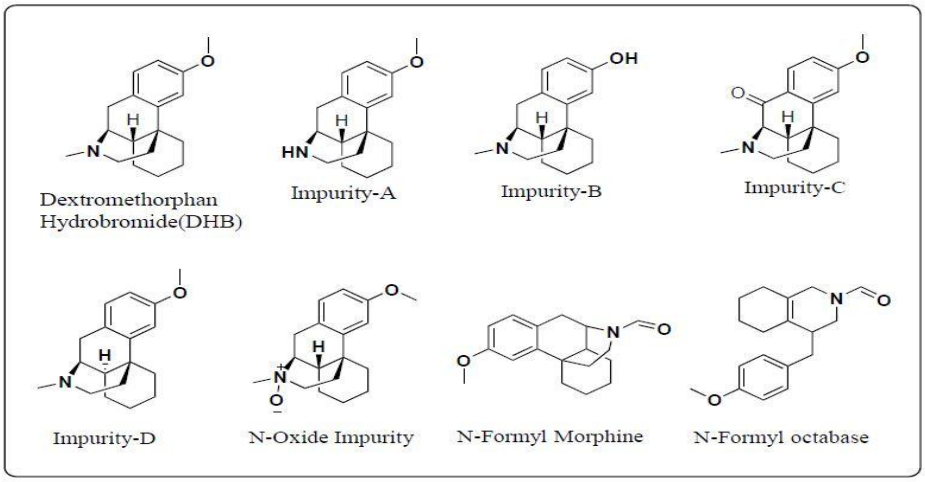

Fig. 1: Chemical structures of DHB and its impurities.

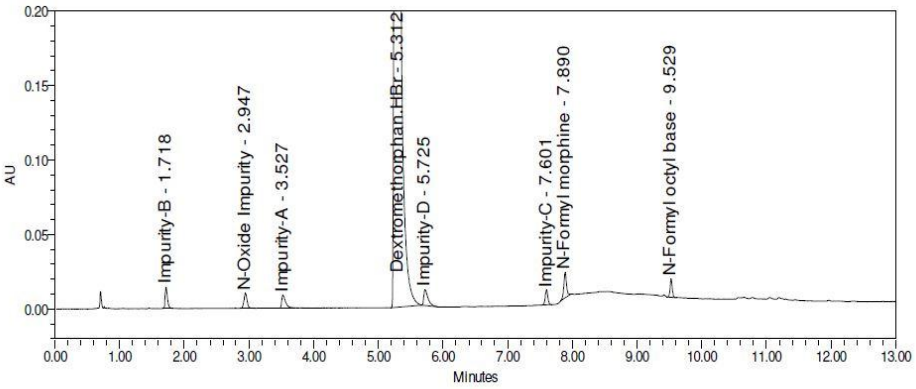

Fig. 2: Chromatogram of DHB (1000 µg mL^{-1}) spiked with 0.2% of all process impurities under optimized Chromatographic conditions.

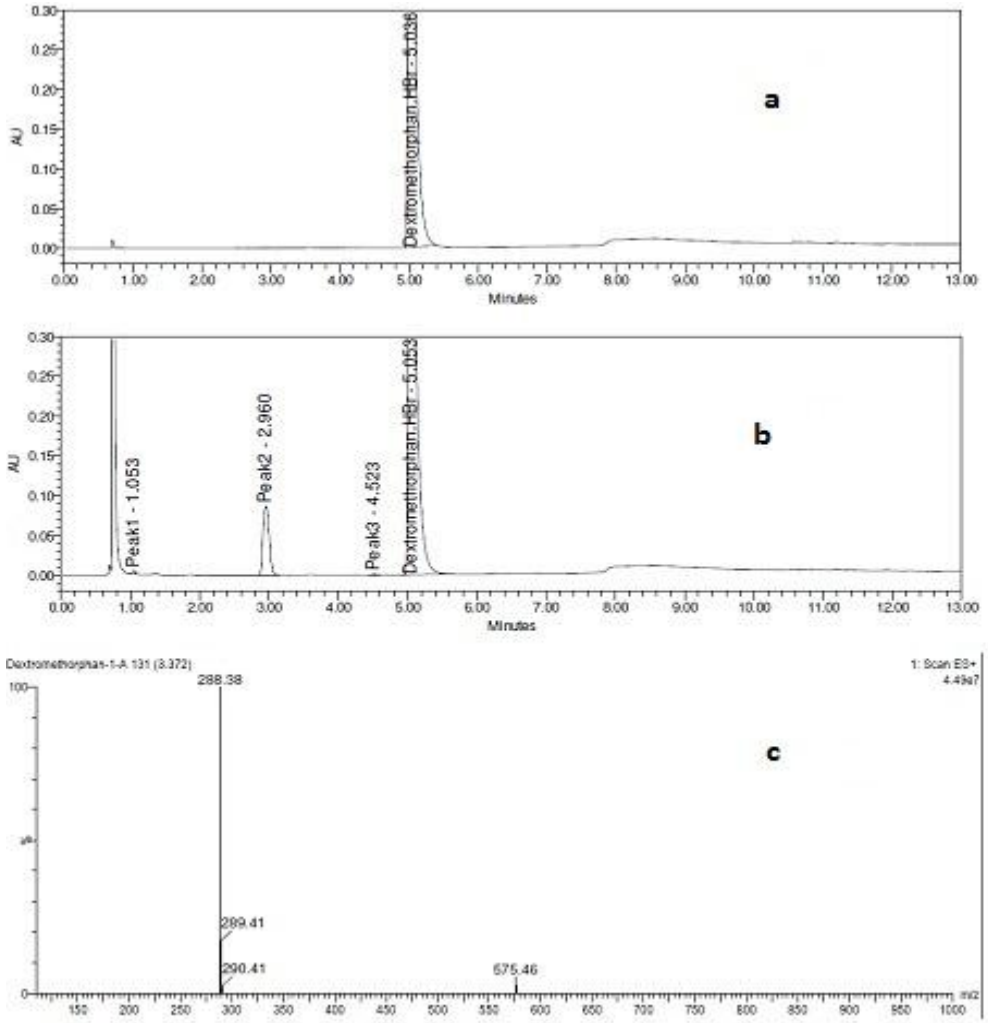

Fig. 3: Chromatograms of DHB Stress samples: (a) As such (b) Oxidative stress sample (c) Mass spectra of Oxidative stress sample.

CONCLUSIONS

The degradation behaviour of DHB was studied under various stress conditions as per International Conference on Harmonization (ICH) prescribed guidelines. Overall one degradation product was formed and characterized by liquid chromatography and tandem mass spectrometry and accurate mass measurements and conformed as DHB N-oxide impurity. A simple gradient reversed phase ultra performance liquid chromatographic method with a low run time of 13 minutes has been developed and validated for the determination of a stability-indicating assay of DHB and its seven related substances in bulk drugs for the first time. The developed method has been found to be selective, precise, linear, accurate, robust, sensitive and rugged. It is applicable for detecting process related impurities and other possible degradation products which may be present at trace levels in bulk drugs. Thus, the method can be used for process development, stability analysis as well as quality control of Dextromethorphan Hydrobromide in bulk drugs.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with the Ethical Standards

The present manuscript does not involve any human participants and/or animal studies and complies with the ethical standards.

Informed Consent

The manuscript is being submitted with the informed consent of all the co-authors.

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