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Response Surface Method Aided Development and Validation of Stability Indicating RP-HPLC-UV Method for Impurities of Dextromethorphan Hydrobromide in API and Three Marketed Formulations

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An innovative, specific, economical and precise method was developed and validated by employing reverse phase high performance liquid chromatography (HPLC) for contemporaneous estimation of dextromethorphan hydrobromide (DHB) along with its impurities in liquid formulation with forced degradation studies and confirmation of content of DHB in composition label in three market formulations along with their impurities were detected by this method. The optimized chromatographic conditions comprised of column (25 cm × 0.46 cm) × 5 µm Kromasil C8 bearing flow rate of 1.5 mL/min and wavelength 220 nm for UV-estimation. Design of experiments were implemented by following Box Behnken design with most optimum parameters selected as follows, column temperature (A), flow rate (B) and pH (C) with corresponding responses comprised of resolution between related compound A (RCA) and DHB (Y_1), tailing of DHB (Y_2) and resolution between related compound B (RCB) and DHB (Y_3). Stress testing was performed and proved that method was stable as no interfering peaks were observed. All validation parameters including suitability, linearity, accuracy, specificity, limit of detection, limit of quantitation, ruggedness, robustness and stress study were evaluated as per updated ICH guidelines and found to be within limit for pure DHB and detected impurities.

Keywords: Dextromethorphan hydrobromide, HPLC, Response surface method, Box Behnken design.

INTRODUCTION

Pure drug, dextromethorphan hydrobromide (DHB) is a dextrorotatory enantiomer of 3-methoxy-*N*-methyl-morphinan, with chemical name as 3-methoxy-17-methyl-9a,13a,14a-morphinan hydrobromide monohydrate (Fig. 1) and comes under the category of drug acting on central nervous system. It is commonly used as a non-opioid anti-tussive drug with high safety assurance in better management of cough and asthma, due to short half-life frequent administration of drug is required to maintain optimum therapeutic level within the body [1-3]. The specified therapeutic dose of DHB has been approved as 20 mg to 30 mg with highest safety assurance, concentration higher than 100 mg was associated with drug abuse, therefore frequently linked side effects includes psychosis, hallucinations and impaired vision [4]. The immense data prevailed through extensive literature sweeping disclosed numerous chromatographic assays for estimation of DHB alone or in multicomponent formulation.

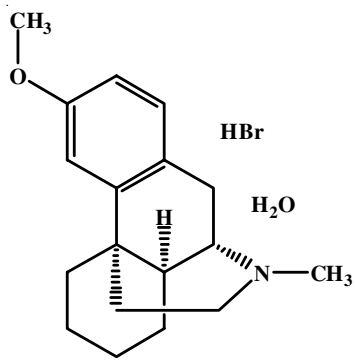

Fig. 1. Structure of dextromethorphan hydrobromide

Din *et al.* [5] developed and validated an uncomplicated, isocratic method for concurrent detection of DHB and chlorph-

eniramine. Chromatographic conditions employed were column dimensions of 15 cm × 4.6 mm with 5 µm particle size and flow rate of 0.8 mL/min at 230 nm UV-estimation [5]. An alternative study conducted by Galli & Barbas [6] for concurrent determination of DHB along with guaifenesin, benzoic acid, saccharin and other components with less than 14 min as the observed baseline by using Discovery C18 column (25 cm × 0.45 cm) at 35 ºC. Poornima *et al*. [7] for contemporaneous determination of DHB and quinidine in bulk and in pharmaceutical tablet dosage form by developing a method employing phenomenex C-8 column with methanol and phosphate buffer (60:40 at pH = 2.5) at 230 nm. As it is well known that in pharmaceutical industry the purity of drug substance and product is highly essential, hence the presence of substance that affects the purity of these results in occurrence of impurities. Impurities are those unwanted chemicals that remain with active pharmaceutical ingredients (API) or were developed during fabrication of dosage form or on ageing of both API and formulation effecting the quality, safety and efficacy. The definition of impurities as per ICH guidelines states that the substances in the API that are not the API itself [8,9]. The estimation of impurities is one of the most tedious task to be executed to maintain safety and quality of drug substance and product, hence several pharmacopoeias had specified a particular limit for presence of impurities [10]. Literature search helped us to explore various chromatographic and spectroscopic techniques utilized in identification and estimation of impurities in API and or drug products. There were few methods available for detection of impurities present in DHB in combination with other drugs, but till date there was no individual method available for contemporaneous estimation of only DHB along with its associated impurities in single method with stability detection. Hence, these above cited data from literature revealed enough justification in furnishing sufficient capability of extensive innovativeness and updated research in the field of pharmaceuticals.

Optimization is a procedure that requires contemporaneous assessment of numerous parameters like composition and type of mobile phase, column temperature, flow rate, retention time, peak intensity, *etc*. so, numerous experimental techniques were applied for HPLC detection of DHB by applying most appropriate experimental design to obtain highly precise and accurate results. The selected experimental design associated in present investigation was Box-Behnken design (BBD) comprising with the software called as design expert 10.0.7.0 version (Stat-Ease Inc., Minneapolis, USA) and the entire procedure obeyed ICH guidelines. The opted parameters in current analysis as per BBD were column temperature (A), flow rate (B) and pH (C) as variables while resolution between RCA and DHB (Y_1), tailing of DHB (Y_2) and resolution between DHB and RCB (Y_3) were taken as response which showed slight variation in flow rate, column temperature and pH affected the response estimated by trails according to factorial design. In existing experimental designed method development we had developed highly novel, accurate, prompt, precise and uncomplicated RP-HPLC method for quantitative analysis of DHB along with its impurities and forced degradation studies in pharmaceutical liquid dosage form (syrup) inclusive of another essential parameter comprising of comparative analysis of DHB in three marketed syrup formulations (Gulfadryl DR 100 mL, Eascof DM 100 mL and Biochemdryl DR 100 mL) for their label claim and along with their associated impurities as per the guidelines stated by in the International council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The developed method was validated by statistical parameters like system suitability, linearity, accuracy, specificity, limit of detection, limit of quantitation, ruggedness, robustness and stress study for pure DHB and selected impurities. The method is highly novel since, till date there is no evidence of detection of DHB and its impurities named as DHB related compound A (RCA), DHB related compound B (RCB) and DHB related compound C (RCC) simultaneously by single gradient method along with content confirmation of three marketed formulations by same method authenticates the entire procedure as highly efficient with inflated innovativeness along with compatibility and making the entire method development process the most lucrative technique available till date. The novelty of this developed method also increases as confirmation of structure of DHB was performed by mass spectroscopy. Hence, it can be used widely for simultaneous estimation of DHB along with its impurities in syrup formulation as well as commonly available marketed preparations for high purity along with compatibility and stress study.

EXPERIMENTAL

Dextromethorphan hydrobromide (DHB) was received as a gift sample from Malladi Drugs Pharmaceutical Ltd., India, butylated hydroxyanisole, propyl paraben and methyl paraben were obtained from Alkem laboratories (P) Ltd, India, potassium dihydrogen phosphate monohydrate, sodium chloride, orthophosphoric acid, triethyl amine, acetonitrile and methanol were received from Merck India Ltd., India.

Waters 2695 HPLC- empower software-3 with 2996 PDA detector was employed in method development process with column comprising of Kromasil C8 bearing dimensions, 250 mm × 4.6 mm with 5 µm particle size. Balance from Mettler Toledo, Lab companion sonicator and pH meter from Lab India were availed in this research.

Chromatographic conditions: The buffer solution A contained 13.6 g potassium biphosphate in 2 L water with 2 mL triethylamine (pH 2.3) and balance regulated with phosphoric acid and mixed well, solution B comprised of methanol. The liquid chromatography with gradient elution was carried out using column Kromasil C8 (250 mm × 4.6mm) 5 µm particle size with optimum flow rate of 1.5 mL/min, 20 µL injection volume, 30 ºC column temperature and 220 nm wavelength for UV-estimation in this developed method. The gradient programme was executed by dividing time (min) with % of solution B (Methanol): 0/40, 18/70, 20/70, 22/40 and 28/40. The preparation of diluent for blank, standard preparation, placebo and for preparation of sample was done by incorporating mixture of 40 g chloride in 1 L water and acetonitrile in the ratio of 70:30 (v/v).

Preparation of stock solution for of impurities: Weighed 12.5 mg of each DHB related compound A (RCA), DHB related

compound B (RCB) and DHB related compound C (RCC) in 100 mL volumetric flask and added 50 mL of prepared diluent, finally dissolved with the help of sonicator.

Standard solution preparation method: Initially, accurately weighed 30 mg of DHB was placed in 100 mL flask then to it 100 mL of diluent was added and placed on sonicator to dissolve completely. In next step, 2 mL of stock solution was placed in 100 mL volumetric flask further diluted to make up the final volume and filtered through 0.45 µ membrane filter.

Sample solution preparation: Accurately weighed 30 mg of DHB was added to 50 mL of diluent and stirred for 60 min, then sonicated for 15 min for complete dissolution. Lastly, the final volume was maintained with diluent after attaining room temperature and filtered with the aid of membrane filter.

Placebo solution preparation: Initially, 12.5 mg methyl paraben, 12.5 mg propyl paraben, 12.5 mg butylated hydroxyanisole and 10 mL of diluent were mixed and sonicated. Then, 2 mL of mixture solution was placed in 100 mL capacity volumetric flask followed by the addition of diluent for final volume and then passed through 0.45 µ membrane filter for filtration.

Experimental design and development of method: The present developed method was designed and optimized for contemporaneous estimation of DHB along with its impurities in syrup formulation as per the suitability of composition of various solvents by varying ratio of buffer mixture, the selected variables were column temperature (A), flow rate (B) and pH (C) as variables while resolution between RCA and DHB (Y_1), tailing of DHB (Y_2) and resolution between DHB and RCB (Y_3). This existing developed method was experimentally designed and optimized by implementation of response surface methodology (RSM) by pertaining BBD with Design Expert 10.0.7.0 software. Implementation of RSM capitulated 17 experimental runs according to specified factors mentioned in Table-1 and using the following polynomial equation for determination of total response variables using following equation:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_1X_1^2 + B_2X_2^2 + B_3X_3^2$$

where, B_0, B_1, B_2 and B_3 were regression coefficient, X_1, X_2 and X_3 were the factors studied and Y was the obtained response corresponding to each factor. Finally, analysis of variance (ANOVA) was implemented to acquire significant difference from obtained matrix design. The values derived on application of ANOVA are presented in Tables 2 and 3, respectively [11-13]. This experimentally designed gradient method employed chromatographic conditions as mentioned earlier with mobile phase comprising of buffer solution A (13.6 g potassium biphosphate in 2 L water containing 2 mL triethylamine) and solution B (methanol). The optimized combination of mobile phase was based upon the data derived from analysis performed consisting of pH range by implementing quality by design, where selection of pH was plotted (Fig. 2). During HPLC method development, log D values are useful in setting of concentration for partitioning of molecules of interest between mobile phase and stationary phase. Log D is the distribution constant that describes lipohilicity of a molecule, specifically used for ioniz-

TABLE-1
REPRESENTATION OF OPTIMIZED CHROMATOGRAPHIC CONDITIONS ALONG WITH THEIR ESTIMATED RANGE

Response	Name	Units	Type	Minimum	Maximum	Low level	High level	Mean	Std. Dev.
A	Temperature	°C	Numeric	25.00	35.00	-1 ↔ 25.00	+1 ↔ 35.00	30.00	3.54
B	Flow	mL/min	Numeric	1.00	2.00	-1 ↔ 1.00	+1 ↔ 2.00	1.50	0.35
C	pH	NA	Numeric	2.00	2.60	-1 ↔ 2.00	+1 ↔ 2.60	2.30	0.20

TABLE-2
THE VALUES OBTAINED ON IMPLEMENTATION OF EXPERIMENTAL DESIGNS FOR ROBUSTNESS STUDY FOR SELECTED FACTORS AND THEIR RESPONSES

Std.	Run	Factor 1 A: Temperature (°C)	Factor 2 B: Flow (mL/min)	Factor 3 C: pH NA	Response 1 Resolution between RCA and DHB NA	Response 2 Tailing of DHB NA	Response 3 Resolution between DHB and RCB NA
16	1	30	1.5	2.3	4.1	0.96	4.6
2	2	35	1.0	2.3	3.7	1.6	4.2
6	3	35	1.5	2.0	3.3	1.4	3.8
13	4	30	1.5	2.3	4.1	0.96	4.6
5	5	25	1.5	2.0	3.4	1.1	3.8
8	6	35	1.5	2.6	3.3	1.5	3.8
1	7	25	1.0	2.3	3.5	1.2	3.9
17	8	30	1.5	2.3	4.1	0.96	4.6
9	9	30	1.0	2.0	3.6	1.3	4.1
12	10	30	2.0	2.2	3.3	1.3	4.0
15	11	30	1.5	2.3	4.1	0.96	4.6
3	12	25	2.0	2.3	2.8	1.8	3.1
4	13	35	2.0	2.3	3.1	1.5	3.5
14	14	30	1.5	2.3	4.1	0.96	4.6
11	15	30	1.0	2.6	3.6	1.4	4.1
7	16	25	1.5	2.6	3.1	1.5	3.4
10	17	30	2.0	2.0	3.3	1.3	3.9

TABLE-3
ANOVA RESULTS FOR RESPONSE

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Prob>F
Obtained data for response Y1 (Resolution between RC A and DHB)						
Model	2.74	9	0.30	48.05	0.0001	Significant
A-Temperature	0.045	1	0.045	7.11	0.0322	
B-Flow	0.63	1	0.63	99.67	0.0001	
C-pH	0.056	1	0.056	8.92	0.0203	
Obtained data for response Y2 (Tailing of DHB)						
Model	1.09	9	0.12	103.28	0.0001	Significant
A-Temperature	0.020	1	0.020	17.05	0.0044	
B-Flow	0.072	1	0.072	61.77	0.0001	
C-pH	0.10	1	0.10	85.43	0.0001	
Obtained data for response Y3 (Resolution between DHB and RC B)						
Model	3.35	9	0.37	57.73	0.0001	Significant
A-Temperature	0.15	1	0.15	23.43	0.0019	
B-Flow	0.64	1	0.64	99.41	0.0001	
C-pH	0.11	1	0.11	16.81	0.0046	

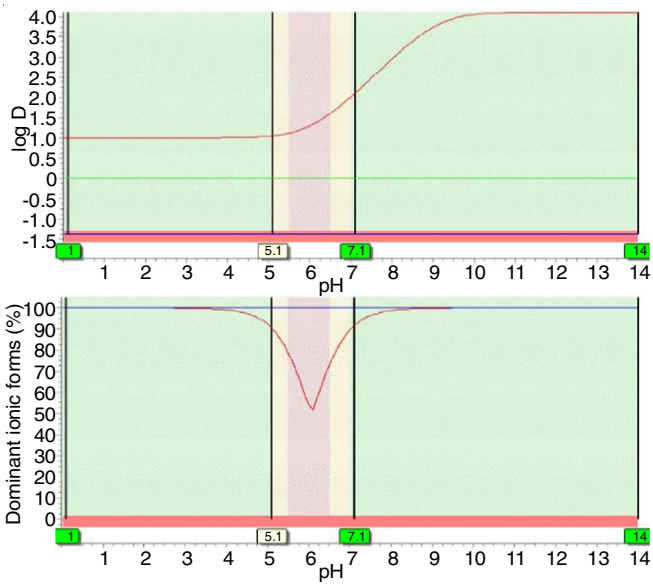

Fig. 2. pH selection for dextromethorphan hydrobromide by quality by design

able compounds due to the pH dependence of a molecule in its aqueous solution. It is estimated as log value by partitioning of a chemical compound allying non-aqueous (lipid) and aqueous phase, so log D refers to the ratio comprising water: octanol partition coefficient at a specified pH value. In present developed method, the parameters pH and log D were selected to estimate lipohilicity of ionizable drug, as it can be seen from graph representing three parts. The green region represents most optimum area, orange represents intermediate area and the red region represents prohibited area for tentative estimation of log D with pH. Detection of suitability of mobile was determined by varying the ratio of combination of buffers with pH maintained at 2.3 with phosphoric acid and methanol involving software. The details obtained after analyzing data helped in selection of most optimized mobile phase comprising of acetonitrile and buffer mixture. System performance was evaluated by verifying all the parameters including system suitability. System precision, relative standard deviation (RSD), tailing of peak, theoretical plates and accurate resolution were calculated.

RESULTS AND DISCUSSION

System suitability: It was determined by injecting six replicate inections of DHB (300 µg/mL) and the data obseved are given in Table-3.

Linearity: Linearity was estimated by preparation of standard solution at varying concentrations. The order was determined from 75-450 mcg/mL for DHB and 0.1% to 1.0% for its impurities. Equation of regression was found to be 0.9999, (x = concentration and y = peak area). The observed values are given in Table-4.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD and LOQ were estimated on the values of standard deviation of response and slope. The observed value for limit of detection for DHB was 0.09 µg/mL for 20 µL injection volume. The observed LOQ for DHB was 0.30 µg/mL, indica-

TABLE-4
REPRESENTATION OF SYSTEM SUITABILITY DATA

Name of compound	Time (min)	Resolution	Tailing	Theoretical plate	Linearity
Impurity C	4.142	NA	0.99	2054	0.99989
Methyl paraben	7.475	9.5	1.02	3567	NA
Impurity A	8.315	4.4	1.47	1985	0.99992
Dextromethorphan hydrobromide	9.228	4.1	0.96	5804	0.99982
Impurity B	10.315	4.6	1.23	2451	0.99988
Propyl paraben	14.142	12.7	1.03	4594	NA
Butylated hydroxyanisol	18.315	17.6	1.12	3917	NA

TABLE-5
PRECISION, LOD, LOQ AND RECOVERY DATA OF DEXTROMETHORPHAN HYDROBROMIDE IMPURITIES

Name	Average precision (%)	RSD (%)	Average of intermediate precision (%)	RSD (%)	LOQ (%)	LOD (%)	Recovery (%)
Impurity-A	0.201	2.5	0.208	2.6	0.015	0.005	96.5
Impurity-B	0.109	2.9	0.101	2.8	0.022	0.007	98.1
Impurity-C	0.099	3.1	0.092	3.3	0.025	0.008	94.4

ting enhanced accuracy with sensitivity in the developed method. LOD and LOQ of impurities are presented in Table-5.

Accuracy: Accuracy was determined by calculating percentage recovery of DHB in sample solution. The observed data are shown in Table-6.

TABLE-6
ACCURACY DATA OF PROPOSED HPLC METHOD FOR DEXTROMETHORPHAN HYDROBROMIDE

Level (%) (n = 6)	Concentration (µg/mL)	Amount recovered (µg/mL)	Recovery (%)	RSD (%)
50	150	148.4	98.9	0.3
100	300	299.1	98.7	0.5
150	450	446.0	99.1	0.4

Precision: The precision was estimated by replication study performed with intermediate precision for prepared syrup formulation of DHB along with its impurities. The data obtained was found to be within the specified range (RSD NMT 2.0%).

Specificity: Specificity was measured by injecting blank, standard, sample, impurities and placebo. It was seen that there was no interference observed because of blank at the retention time of DHB. The chromatogram showed identical peaks for placebo and sample without overlapping of any extraneous peak at the retention time.

Analytical solution stability: It was examined by preparation of standard and sample solution as per guidelines stated for preparation further they were injected at specified time intervals for 24 h, finally calculted cumulative % RSD for peak areas of DHB. No changes in assay values were observed for 24 h exhibiting stability of drug in the solvent. The data obtained for estimated impurities along with detection of impurities in marketed formulations are reported in Table-7.

Stress testing: Degradation of drug was determined by executing stress studies as per ICHguidelines by performing forced degradation studies employing mass balance.

Acid-base degradation: This tesing was performed to degrade a drug substance forcefully into its initial degradation products by exposure of extreem stress conditions of acidic and alkaline nature for stipulated time period. Acid testing was initiated by taking samples and reacting with 5.0 mL of 1.0 N HCl then refluxed at 80 ºC for 60 min, finally, the treated sample was observed and recorded. Alkali (base) degradation testing was executed by reacting sample separately with 5 mL of 1.0 N NaOH then refluxed at 80 ºC for 60 min, finally, the treated samples were observed and recorded in Table-8.

Thermal degradation: It was executed by placing sample solution for 4.0 h at 60 ºC, then it observed for its degradation products by implementing developed HPLC method and finally the obtained data was recorded and shown in Table-8.

Oxidative degradation: Oxidative degradation was executed by treating drug sample with 5 mL of 3.0%v/v solution H_2O_2 at 80 ºC for 60 min. Finally, H_2O_2 treated sample was observed and data recorded in Table-8.

Photostability: Pharmaceutical drugs and drug substsnces were mostly exposed to highly energetic electromagnetic radiations like ultraviolet and visible light. The drug sample and its control were exposed to cool white fluorescent and near ultraviolet lamp. The observed data obtained from degradation studies of drug revealed that no major unknown degradation was found, the resultant photo-degraded samples were in the range of 1.4% to 4.3% in DHB (Table-8). The data observed

TABLE-7
COMPARATIVE DATA STUDIED WITH MARKATED FORMULATION

Samples	Dextromethorphan hydrobromide (assay %)	Impurity-A (%)	Impurity-B (%)	Impurity-C (%)
Gulfadryl DR 100 mL syp	96.1	0.304	0.201	0.157
Eascof DM 100 mL syp	95.7	0.310	0.215	0.145
Biochemdryl DR 100 mL syp	95.3	0.319	0.208	0.136

TABLE-8
STRESS TESTING DATA FOR DEXTROMETHORPHAN HYDROBROMIDE

Type	Condition	Period	Purity angle	Purity threshold	Assay (%)	Drug degradation (%)
Control sample (As such)	No treatment	–	0.084	0.251	96.9	NA
Acid degradation	1.0 N HCl (80 °C)	60 min	0.084	0.245	92.6	4.3
Base degradation	1.0 N NaOH (80 °C)	60 min	0.068	0.257	95.5	1.4
Peroxide degradation	3.0% H_2O_2 at 80 °C	60 min	0.079	0.255	95.2	1.7
Thermal degradation	60 °C	4 h	0.069	0.247	93.8	3.1
Photolytic degradation	254 nm	2 h	0.078	0.255	93.9	3.0

data exhibited that developed method was specific for estimation of DHB. The obtained data is represented in Fig. 3.

Robustness: It was determined by BBD with three selected dependent variables and simultaneously three corresponding responses were analyzed with 17 runs followed as robustness parameters. The maximum chance of occurrence of possibilities were observed, detected and noted in Tables 1 and 2. Implementation of Box Behnken design (BBD) yields various models, based on the suggested models execution of several systems, responses like resolution between RCA and DHB (Y_1), tailing of DHB (Y_2) and resolution between DHB and RCB (Y_3) were observed. Influence of uninhibited parameters

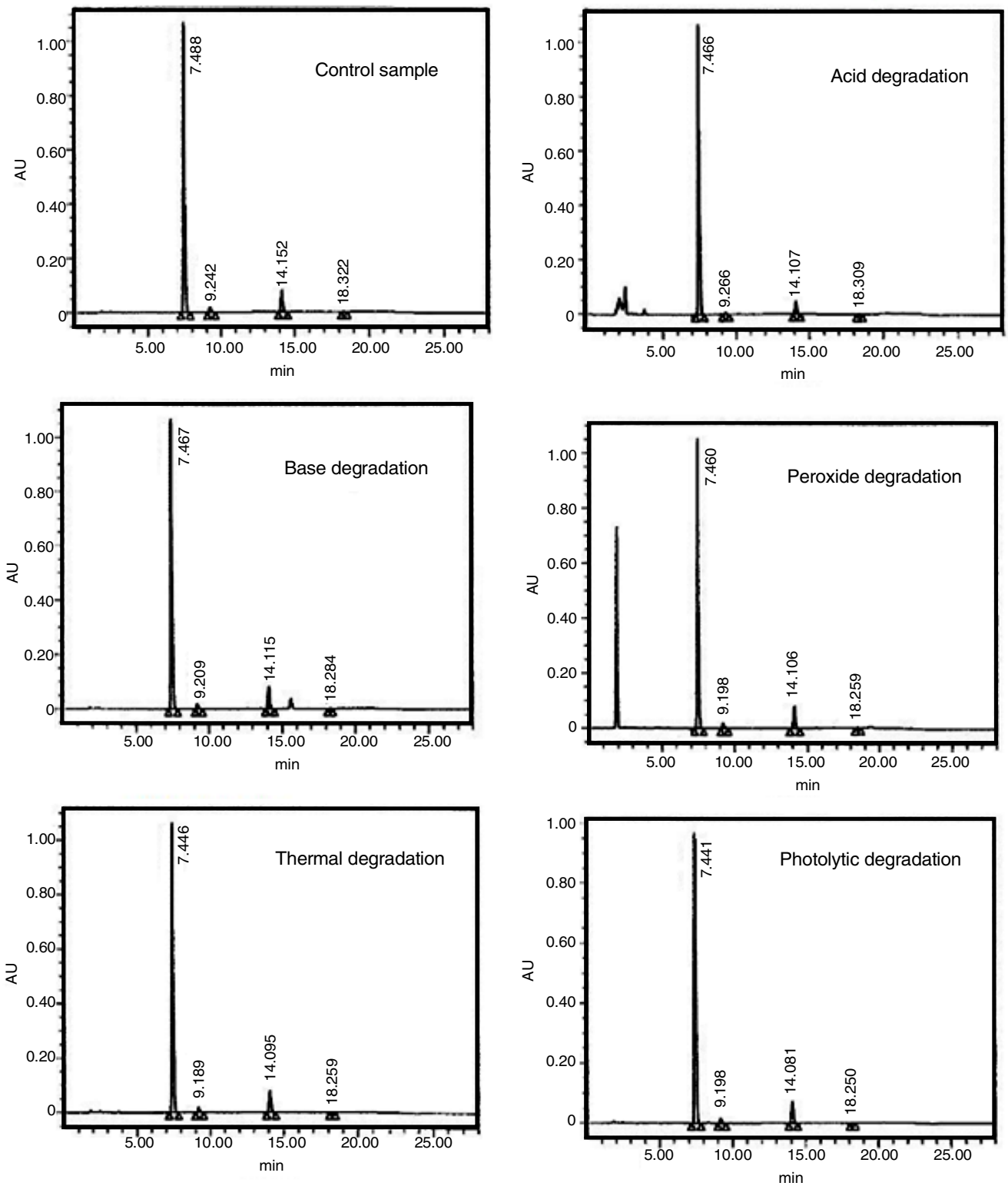

Fig. 3. Stress testing chromatograms of dextromethorphan hydrobromide

curtailed to avert unwanted effect on response, so randomized method was opted for every individual experiment. Lastly, the observed data and contour plots along with 3D surface studies obtained through implementing ox Behnken design showed enhanced accuracy and precision for observed data as can be co-related with Figs. 4-6.

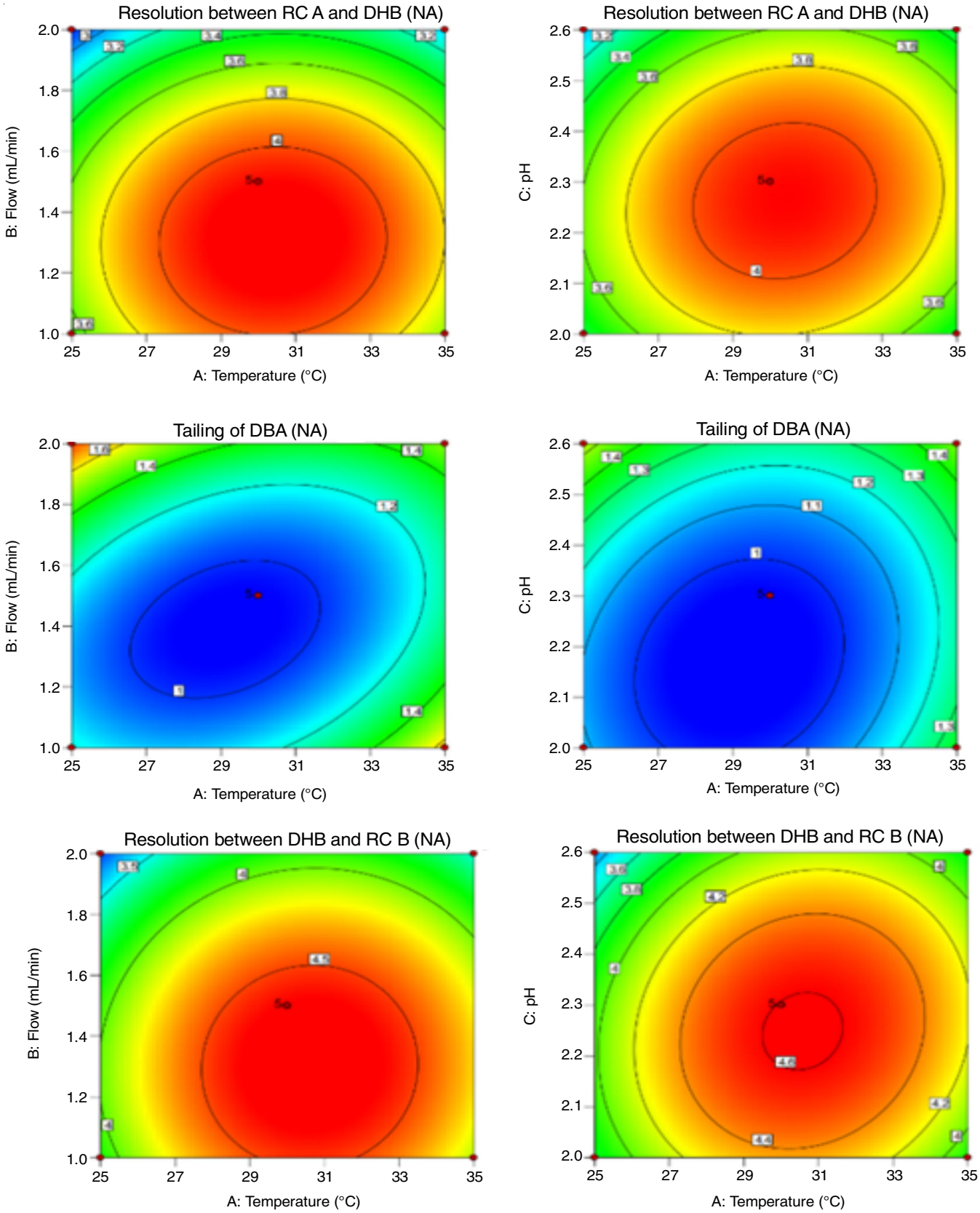

Fig. 4. Contour plots effect of factor A, B and C on responses Y_1, Y_2 and Y_3

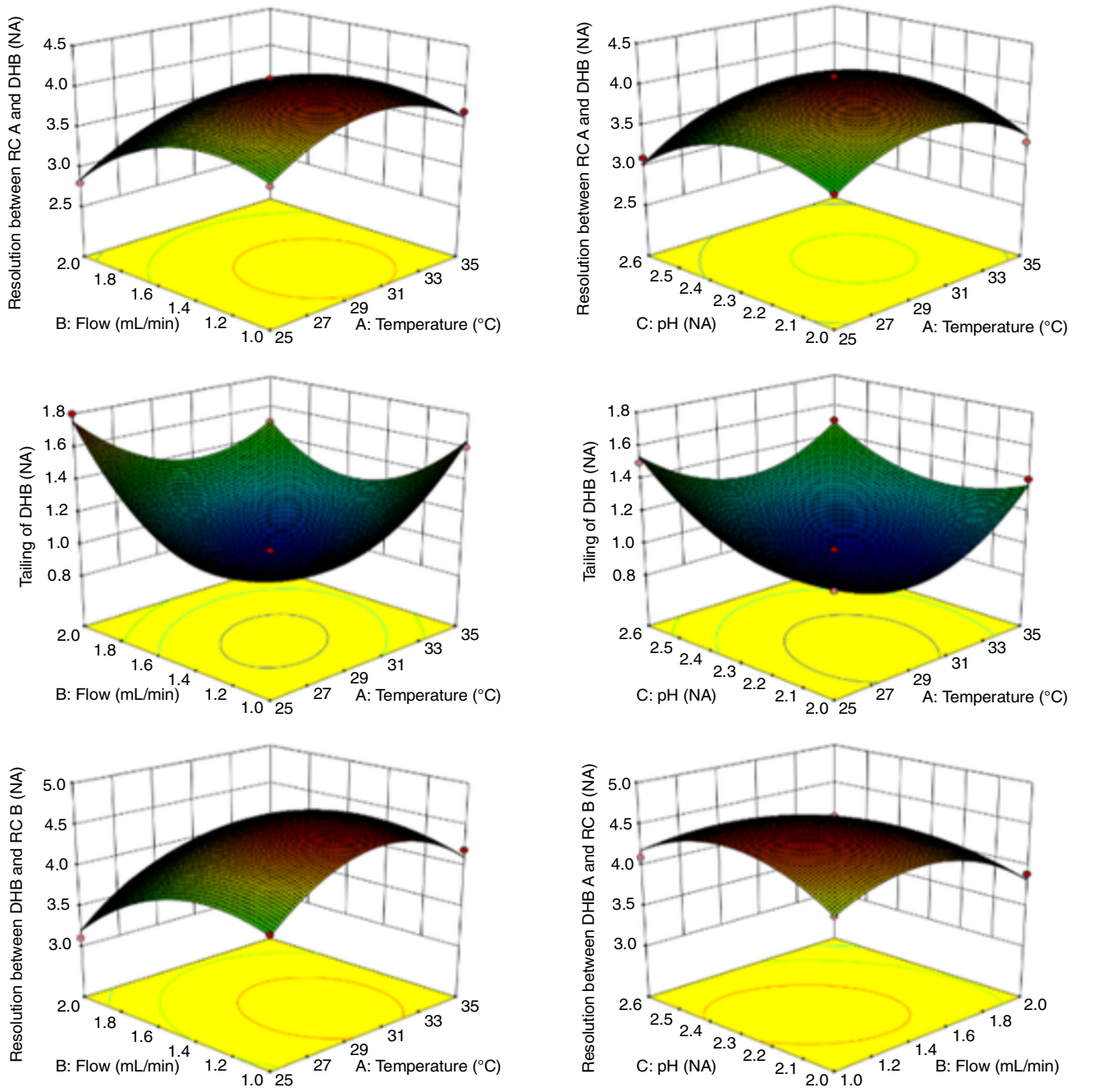

Fig. 5. Three dimensional response surfaces effect of factor A, B and C on responses Y_1, Y_2 and Y_3

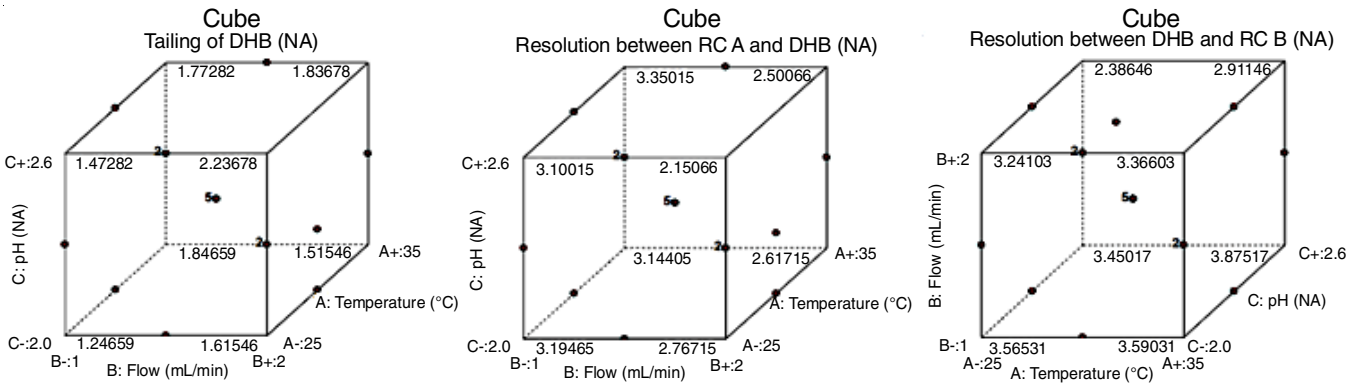

Fig. 6. Cubic response surfaces effect of factor A, B and C on responses Y_1, Y_2 and Y_3

Structural confirmation by mass spectroscopy: The data obtained from LC-MS Waters instrument detection revealed that protonated ions $(M+H)^+$ of dextromethorphan is 272.23 Da in Fig. 7.

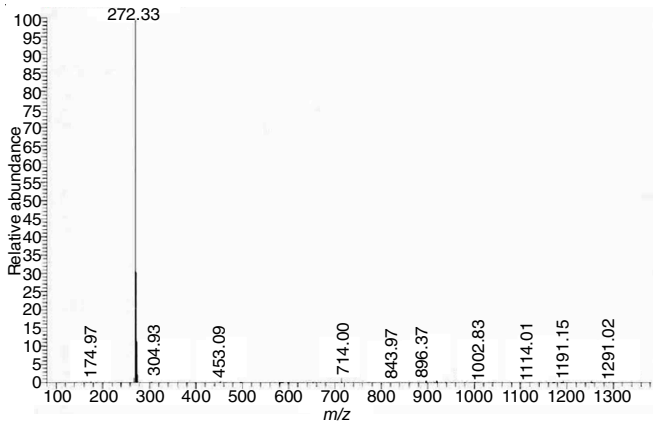

Fig. 7. MS spectra of dextromethorphan hydrobromide

The method exhibited appropriate preparation along with lesser retention time and maximum impurities detection during analysis. The parameters comprising column temperature, flow rate and pH depended upon system suitability, linearity, accuracy, sensitivity and forced degradation study. The various combinations obtained from BBD recommended the most optimum concentration of solution A and B maintained at pH 2.3 for presently designed gradient programme with 1.5 mL/min flow rate at 30 ºC column temperature and 220 nm wavelength, representing excellent chromatographic separation of DHB and its impurities (Fig. 8). The described method has been validated for the assay of DHB using the following parameters. The comparative study with marketed formulations were performed along with their impurities detection with maximum accuracy with the help of this single method and the observed data was recorded.

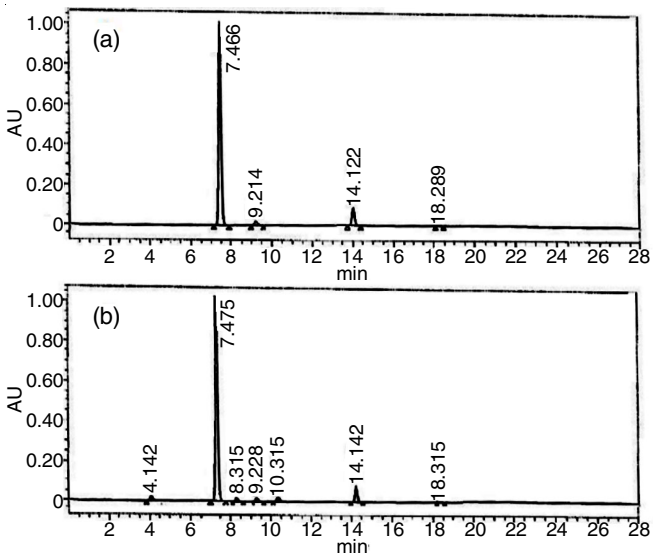

Fig. 8. Chromatogram of dextromethorphan hydrobromide with placebo (a) and its impurities (b)

Conclusion

The gradient method developed and validated was highly innovative, simple, cost effective and convenient as it can be used with high efficiency and accuracy for contemporaneous estimation of dextromethorphan hydrobromide (DHB) with its impurities along with content confirmation of drug in following mentioned marketed formulations (Gulfadryl DR 100 mL, Eascof DM 100 mL and Biochemdryl DR 100 mL)) with their impurities. Hence, this single method can be widely used for multiple estimation that is in active pharmaceutical ingredients and in any available dosage form by following ICH guidelines. The observed data for validation parameters confirmed inter and intra day variations within specified limit as precision data was less than 2% for RSD. The proposed method was determined as specicfic, selective, precise, stable, reproducible and accurate.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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