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LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF DESLORATADINE AND ITS METABOLITE 3-HYDROXY DESLORATADINE IN HUMAN PLASMA

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ABSTRACT

An accurate and highly sensitive LC-tandeem mass spectrometric method has been developed and validated for the quantification of desloratadine and its metabolite 3-hydroxy desloratadine according to regulatory guidelines. Following liquid–liquid extration analytes and internal standards (ISs) were extracted from K_3EDTA human plasma. Chromatographic separation was achieved on Kromasil C_{18}column (150mm x 4.6mm I.D, 5µm particle size) using a mixture of Methanol: Acetonitrile (60:40V/V): 10mM Ammonium formate, (70:30V/V) as the mobile phase at an isocratic flow rate of 1.0 mL/min. The precursor and product ion transitions monitored in multiple reaction monitoring (MRM) mode via turbo ion spray source operating at positive mode. The mass transitions of desloratadine, 3-hydroxy desloratadine and their internal standards desloratadine-D_4, 3-Hydroxy desloratadine-D_4 were m/z 311.10 →259.20, 327.10→275.10, 315.20→263.20, 331.10→279.10 respectively. The method was linear over the concentration range of 50.0pg/mL to 10000pg/mL with correlation coefficients a lower limit of quantification (LLOQ QC) of 50.0pg/mL. The intra and inter day precisions and accuracy were < 5.71, < 5.47, < 5.10, < 6.68 and -6.67 to 5.00, -7.80 to 2.60, -4.00 to 3.75, -6.00 to -0.25 respectively. The proposed method was successfully applicable in vast majority of pharmacokinetic study of drugs.

KEY WORDS

Desloratadine, 3-hydroxy desloratadine, Plasma, LC-MS/MS

INTRODUCTION

Desloratadine (DES) is active metabolite of loratadine, the advantage of the second generation H_1 antihistamines is their non-sedating effect on the central nervous system which is observed with first generation H_1 antihistamines because of crossing the blood brain barrier due to their high lipophilicity. Its chemical name is 8-Chloro-6, 11-dihydro-11-(4-piperidinyidene)-5Hbenzo [5,6]cyclohepta [1,2,b] pyridine and has a structure as below (Figure1). Desloratadine has a long-lasting effect and does not cause drowsiness because it does not readily enter the central nervous system. Unlike other antihistamines, desloratadine is also effective in relieving nasal congestion, particularly in patients with allergic rhinitis. The molecular formula of desloratadine is $C_{19}H_{19}ClN_2$ with a molecular weight 310.82 gms/mole. Desloratadine (a major metabolite of loratadine) is extensively metabolized to 3-hydroxy desloratadine (3-OH DES) (8-Chloro-6,11-dihydro-11-(4-piperidinylidene)-5H-benzo[5,6]cyclohepta[1,2-β]pyridin-3-ol), an active metabolite, which is subsequently glucuronidated. Approximately 87% of a 14C-desloratadine dose was equally recovered in urine and feces.

Several methods have been reported for quantification of desloratadine in human plasma with LC-MS[1], Zhang YF *et al*[5] developed a LLE method over

a concentration range of 0.1-20ng/mL. Among all researchers, L. Yang *et al*[12] and Shen J X *et al*[13]achieved the best results with high sensitivity at a linearity range with SPE extraction. Xu *et al*[13] reported the simultaneous determination of desloratadine and its metabolite, 3- hydroxyl desloratadine in human plasma by LC-MS/MS.

The objective of proposed method is to extract desloratadine in human K_3EDTA plasma by LLE method high sensitivity and selectivity along with its metabolite appreciably compared with the deuterated internal standards (ISs), which is most appropriate in terms of matrix effect, recovery and reproducibility. The developed method could be applicable for conducting BABE studies to various regulatory bodies.

MATERIALS AND METHODS

Chemicals and materials

Desloratadine, 3- hydroxy desloratadine and desloratadine-D_4, 3-hydroxy desloratadine-D_4 [Internal Standards (ISs)] were purchased from Samex Overseas, Surat-1, India and Toronto Research Chemicals Inc., Canada respectively. HPLC grade acetonitrile, methanol were procured from Thermo Fisher Scientific India Private Limited (Sion East, Mumbai, India), Blank (drug-free) human plasma in K_3EDTA was obtained from Supratech voluntary blood bank, Thaltej, Ahmedabad. Water was purified by a Milli-Q system for the preparation of samples and buffer solution. Formic acid AR grade, sodium hydroxide (pellets) GR grade, dichloromethane HPLC grade, ammonium formate GR grade, diethyl ether AR grade were produced from Merck (Worli, Mumbai, India).

Solutions

10 mM ammonium formate was prepared appropriately. Organic mixture was prepared by the addition of acetonitrile, methanol in the ratio of 40:60 V/V, to this mixture added 10 mM ammonium formate in the ratio of 70:30(V/V) to compose mobile phase. Diluent solution was prepared with 0.1% formic acid and methanol in the ratio of 50:50 (V/V). Sodium hydroxide solution was prepared appropriately in water. Diethyl ether and dichloromethane for extraction purpose in 70:30 (V/V) ratio.

Stock solutions

Stock solutions of desloratadine and 3-hydroxy desloratadine were prepared in the diluent solution that gives 100µg/mL concentration separately. These stock solutions were used for preparation of spiking solutions required. A mixed intermediate solution 2.5µg/mL concentration contains desloratadine and 3- hydroxyl desloratadine was prepared. Prepared mixed intermediate solution used to spike calibration standards (CC) and quality control standards (QC) of desloratadine and 3 hydroxy desloratadine. The CC and QC standards were used to evaluate accuracy and precision. It was also used for the determination of lower limit of quantification (LLOQ). Stock solutions of desloratadine-D_4, 3-Hydroxy desloratadine-D_4 having 100µg/mL was used as IS working solution.

Instrument and LC-MS/MS conditions

The HPLC system (Shimadzu, Kyoto, Japan) equipped with LC-20AD Series pumps used for solvent delivery, DGU-20 A3 degasser, CTO-AS vp column oven and a high throughput SIL HTc auto sampler system was used for analysis. The analytical column was Kromasil C_{18} column (150mm x 4.6mm I.D, 5µm). Mobile phase composed with organic mixture (acetonitrile: methanol, 40:60 V/V): 10mM ammonium formate 70:30 (V/V). Separation was achieved under isocratic elution condition at flow rate was 1.0 mL/min, 75% flow splitting. In-order to found the tuning parameters for analytes and internal standards 100ng/mL concentration solution were prepared in mobile phase system, infused separately at a flow rate of 10µL using Hamilton syringe pump and scanned parent, product ion masses in full scan mode recorded in the range of 50 to 400 amu. Nitrogen was used as nebulizer gas 50.00 L/h, zero air as sheath gas and the resolution was set at unit mass. Mass spectrometric detection was performed using an API 4000 triple quadrupole instrument (MDS-SCIEX, Toronto, Canada) and on multiple reaction monitoring (MRM). A turbo ion spray interfaced in positive ion mode with unit resolution was used. The MRM transitions monitored were 311.10 (m/z) →259.20 (m/z), 315.20 (m/z) →263.20 (m/z) for desloratadine, desloratadine-D_4 respectively. Similarly 327.10 (m/z) →275.10 (m/z), 331.10 (m/z) →279.10 (m/z) for 3-

hydroxy desloratadine, 3-hydroxy desloratadine-D_4 respectively with a dwell time set to 200 ms for each selected ion transitions. Analyst software version 1.4.1 and Watson LIMS version 7.3 were employed for data acquisition and processing.

Sample preparation for LC- MS/MS analysis

To evaluate the strategy and mimic the types of samples one would analyse with this approach, a set of a drug and metabolites were spiked into plasma an aliquot of 500 µL plasma, 50 µL of internal standard mix (25 ng/mL Desloratadine-D_4 & 3-hydroxy desloratadine-D_4), and 50µL of 0.1 Normal/L sodium hydroxide solution were added. The samples were vortex-mixed then centrifuged and extracted with 2.5 mL of diethyl ether-dichloromethane (70:30 V/V) at 4000 rpm/ 2min, 10±2°C. The organic layer was separated and evaporated to dryness at 40 ± 5°C under a gentle stream of nitrogen. The residue was dissolved in 100µL of mobile phase [Organic Mixture: 10mM ammonium formate, (70:30 V/V)] and vortex mixed. A 10µL aliquot of the solution was injected into the LC-MS/MS system.

Method validation

The method was validated and applicable to current regulatory guidelines. The selectivity of the method was evaluated by analysing 10 different types of plasma, which included 7 K_3EDTA and one each of lipidemic, heparinised and haemolysed plasma. Interference of commonly used medications by human volunteers was checked in six different batches of plasma having K_3EDTA as anticoagulant. Their stock solutions were prepared appropriately in methanol, spiked in plasma and analzyed under the same conditions at LQC and HQC levels in six replicates. These sets were processed along with freshly prepared calibration standards and QC standard samples in duplicate.

The linearity of the method was ascertained by measuring the peak area ratio response (analyte/IS) for eight non-zero concentrations. Each of them was analysed by using least square weighted ($1/x^2$) linear regression.

The extraction recovery for analytes and ISs was calculated by comparing the mean area response of extracted samples with that of un-extracted samples at HQC, MQC and LQC levels. Matrix effect was assessed by comparing the mean area response of un-extracted samples with mean area of standard solutions at three QC levels. Percentage recovery for drug and analyte were represented in Table1 (a) & (b). Matrix ion suppression was conducted by post column infusion of analytes (MQC level) at 10µL/min through a 'T' connector.

Stability was determined by measuring the area ratio response (analyte/IS) of stability samples against freshly prepared comparison standards at LQC and HQC levels. Stock and working solutions of analytes and ISs solutions were checked for short term stability at room temperature and long term stability. Autosampler stability (wet extract). Bench top (room temperature) stability and freeze-thaw stability were determined at LQC and HQC levels using six replicates for each level. Long term stability of spiked samples was also examined for both QC levels.

Ruggedness of the method was evaluated on precision and accuracy batches. The first batch was analyzed by different analyst and second batch was on two different equipment and column. Dilution integrity was determined by diluting the stock solutions two fold and tenfold for desloratadine and 3-hydroxy desloratadine in screened plasma. The accuracy and precision for dilution integrity standards at HQC and LQC were performed the sample analysis against freshly prepared calibration standards.

Matrix effect is the suppression or and enhancement of ionization of analytes by the presence of matrix in the biological samples and quantitative measure of the matrix effect termed as matrix factor. Evaluated by spiking the analytes and ISs at QC levels of higher and lower into analyte free plasma from six different batches of respective matrix and in duplicate along with six replicates of aqueous samples at higher and lower QC levels the same. The matrix factor was measured as coefficient of variation less than 15%.

RESULT AND DISCUSSION

For development of liquid chromatography method, solubility of desloratadine and its metabolite 3-hydroxy desloratadine was studied in order to determine the proper ratio of solvents used as mobile phase to the drug substance for analysis. Referring to chemical structure, the compound is a base and is able to accept proton(s), therefore polarity of the

dissolved solvent will affect the solubility. The feasibility of several mixtures of solvent such as acetonitrile and methanol using different buffers such as ammonium acetate, ammonium formate, acetic acid and formic acid with variable P^H range 3–6 was tested for complete chromatographic resolution. To optimize the chromatographic conditions, different combinations of methanol–water (90:10,70:30, 50:50, 40:60, 30:70 V/V), methanol-acetonitrile (80:20, 60:40, 50:50, 40:60 V/V), and acetonitrile–ammonium formate buffer (90:10, 80:20, 60:40, 50:50, 30:70 V/V) were tested. The effect of mobile phase strength on chromatography of the analytes was evaluated by changing the organic solvent to aqueous buffer ratio. The mobile phase composition resulted in good response and peak shape was selected as the mobile phase. Acetonitrile and methanol with ammonium formate buffer (P^H 3.5) was preferred because it resulted in a greater response to desloratadine and its metabolite 3-hydroxy desloratadine after several preliminary investigatory runs compared with the other mobile phases. Poor resolution of desloratadine and its metabolite were observed using ammonium formate-methanol (60:40 V/V) but better results were achieved with (40:60 V/V) of ammonium formate–organic mixture (made of binary mixture containing Acetonitrile and methanol). In terms of buffer concentration, 5 mM and 10 mM ammonium formate buffers were investigated. On comparing the peak areas (response) for desloratadine and 3-hydroxy desloratadine using these two buffers, it was concluded that the 10 mM ammonium formate buffer solution proved considerably better for the peak shape and area of the drug

Method development

Method development is initiated with the scanning for parent and product ions using 100ng/mL solution analytes and with internal standards of both in the positive and negative polarity modes between m/z 50 and 400 range. However, it was difficult to get the deprotonated precursor ions for analyte and its metabolite in negative mode and hence positive ionization mode was selected. Apart this the intensity found was much higher in the positive ion mode for desloratadine and 3-hydroxy desloratadine and the internal standards as they have similar sites for de-protonation. Mass scanning was done in the range of 50 to 400 amu. Analytes of interest have high sensitivity in electrospray ionization[7] mass spectrometric analysis compared to non-polar and un-ionisable organic compounds, due to their polar nature and presence of highly ionisable functional groups such as amine or carboxylic acid in their structures. Further, the use of 10mM ammonium formate in the mobile phase enhanced the response of de-protonation precursor $[M+H]^+$ ions. Turbo ion spray interface[7] (TIS) operated in positive ionization mode was used for the detection. A highly stable and intense precursor ion was formed at MRM transitions monitored were m/z 311.10 (m/z), 315.20 (m/z), 327.10 (m/z) and 331.10 (m/z) in the Q1 MS full scan spectra for desloratadine, desloratadine-D_4, 3-hydroxy desloratadine, 3-hydroxy desloratadine-D_4. Similarly the most abundant product ions at m/z 259.20 (m/z), 263.20 (m/z), 275.10 (m/z) and 279.10 (m/z) in the Q1 MS full scan spectra for Desloratadine, Desloratadine-D_4, 3-hydroxy desloratadine, 3-hydroxy desloratadine-D_4 were found by optimizing collision energy with a dwell time of 200 ms per transition and the quadruples 1 and 3 were set at unit resolution. The MRM state file parameters like nebulizer gas, CAD gas, ion spray voltage and temperature were suitably optimized to obtain a consistent and adequate response for the analytes and internal standards. Optimised parameters for both ion source and compound dependent parameters were represented in Table 3(a) & (b).

Optimization of chromatographic conditions

LC conditions for analytes and ISTDs were set under isocratic mode and gradient analysis was deliberately avoided without compromise in the run time and chromatography. Acidic mobile phase has improved the protonation of all compounds and addition of buffers even at 10mM concentration has shown a positive impact on signal intensities. Highly significant response variation was observed between acetonitrile and methanol in the mobile phase, however consistent and reproducible results were obtained with binary mixture of acetonitrile and methanol. During the optimization of stationary phase several columns were checked and better chromatography with good improved peak responses was observed on Kromasil-C_{18} basic column. A flow rate of 0.8mL/min was used to minimize the run time and a post column

with splitness of 25:75 was used without compromise in the signal intensity.

Choice of Stationary Phase

Three analytical columns and various mobile phase compositions were tried in order to reach an acceptable separation as well as a reasonable chromatographic run time. For organic semi-polar molecules, sample retention decreases with increasing length of the bonded phase. Desloratadine and its metabolite 3-hydroxy desloratadine are basic polar compounds and freely soluble in water and acetonitrile mixture, were eluted rapidly from the C_{18} column. The cyano (CN, Phenomenex, 250 mm × 4.6 mm I.D, 5-µm particle size) chromatographic column is used for polar basic compounds in both reverse and normal-phase modes. A cyano-column was highly retentive for the analytes, and thus resulted in late eluting peaks especially for desloratadine (Retention time >20 min). Retention of desloratadine and its metabolite 3-hydroxy desloratadine on cyano (CN) was greater than on the other columns (C_{18}), with good chromatographic response and optimum separation, but peak shape was not optimum. On the basis of these findings, the C_{18} analytical column was selected as most appropriate for analysis of desloratadine. And different columns were used including a Kromasil C_{18} column (250mm×4.6mm I.D, 5 µm particle size; and a Diamonsil C_{18} column (250mm×4.6mm I.D, 5 µm particle size). Finally, Kromasil C_{18} has selected for its excellent system suitability parameters and symmetrical peaks and with good resolution between the two analytes.

Selection of Internal standard

The internal standard was selected on the basis of chemical structure, polarity and solubility characteristics. Compounds with close pKa and log P values were selected as ISTDs, to nullify the variations in sample preparation step, as it is the most susceptible area for variations. According to the internal standard selection guidelines, the internal standard should ideally mirror the analytes in as many ways as possible. It should preferably belong to the same class, with same physicochemical and spectral properties to significantly improve the method ruggedness[9]. Different drugs like imipramine and mefenamic acid were tested as potential candidates for internal standard and finally, deuterated internal standard superseded all other candidates in terms of consistency and reproducibility. There was no significant effect of internal standards on analytes recovery, sensitivity or ion suppression. Moreover, there was no matrix effect of internal standards on desloratadine and 3-hydroxy desloratadine. Also, the validation results obtained from this LC-MS/MS method encouraged its selection as internal standards for the present study.

Optimization of extraction procedure

Initially, the extraction of desloratadine and 3-hydroxy desloratadine was carried out by protein precipitation with common solvents like acetonitrile, methanol and the obtained results were not satisfactory with respect to the sample clean-up as the solvents usable for the protein precipitation are not strong enough to clean up the sample fully and this may lead to column clogging, source contamination and finally contamination to the mass detector. Phospholipids are major components that stay in the extracts and elute late in the columns. It may not affect polar compounds as much as they do for late eluting analytes. However, the lipids require high organic composition in the LC gradient to clean them out of the LC column, otherwise it will accumulate inside the column and cause poor peak shape or retention shifts. Liquid-liquid extraction technique was also tested to isolate the drugs from plasma using diethyl ether, dichloromethane, methyl tertiary butyl ether, ethyl acetate and dichloromethane (alone and in combination) as extracting solvents. With LLE, the majority of phospholipids stay in the aqueous layer when using MTBE as an extraction solvent regardless if the plasma P^H is adjusted or not. By switching to a more polar organic extraction solvent, such as dichloromethane, more lipids will participate into the organic layer. Diethyl ether: Dichloromethane, 70:30V/V in ratio as an extraction solvent mix gives best results over the other solvents mix. The phospholipids response increases ~ 10 fold in the organic layer compared to using only MTBE. This suggests using Diethyl ether: Dichloromethane, 70:30V/V in ratio as an extraction solvent in the application of analyzing the aqueous layer in LLE.

Method Validation

Validation[10,11] runs were conducted on six separate days. Each precision and accuracy validation run organized with a set of spiked calibration standard samples distributed at eight concentration levels over the dynamic range, a blank (without ISTD), a zero sample (blank with ISTD) and QC samples (n=6 at each of four concentration levels; LLOQ QC, low, medium and high). Standard samples were analyzed at the beginning of each validation run and other samples were distributed randomly throughout the run. Results of the QC samples from four inter &intraday runs were used to evaluate the accuracy and precision of the method. Sensitivity (at lower limit of quantification), dilution integrity (two fold and tenfold), and ruggedness of the method were also determined.

Selectivity: Selectivity is the ability of the method to measure and to differentiate the analyte in the presence of endogenous components, which may be expected to be present, typically the endogenous components might include metabolites, impurities, matrix components, etc. The selectivity of the method towards endogenous plasma matrix was ascertained in ten batches of human plasma by analyzing blanks and spiked plasma samples at LLOQ QC concentration. No endogenous peaks were observed at the retention time of the analytes for any of the batches. Method selectivity is established by proof of the lack of response by analyzing human K_3 EDTA plasma blank matrices from ten different individual matrix lots along with one lipemic, one heparinized and one hemolytic plasma lots. Peak responses in blank plasma lots were compared against the mean response of spiked LLOQ QC samples (n=6) and no interferences were observed in all the screened plasma lots, at the retention time of analytes and ISTDs. The selectivity of the method, with the chromatograms of blank plasma and standard zero with internal standards demonstrated at Figure 2 respectively. The linear regression of the peak area ratios versus concentrations were fitted over the concentration ranges 50.0pg/mL to 10000pg/mL of the analytes in plasma.

Specificity sensitivity

Method sensitivity was determined in one of the validation runs at LLOQ QC level by perform and processing six replicates. The batch precision at LLOQ QC level was < 5.71, <5.47, for DES, IS and< 5.10, < 6.68 for 3-OH DES and IS and while accuracy -6.67 to 5.00 for DES -7.80 to 2.60for DES-D_4, -4.00 to 3.75 for 3-OH DES, -6.00 to -0.25 for 3-OH DES-D_4 respectively. Precision and accuracy for the proposed method was represented in Table 2. No endogenous compounds were found to interference at the retention time of analytes and ISs. The representative chromatogram of a calibration standard at the LLOQ QC level is shown in Figure 3.

The precision (%CV) for system suitability test was in the range of $\leq$ 1.65 & $\leq$ 1.75 for the retention time and for the area ratios of both analytes and ISs. The signal to noise ratio for system performance was ≥5.5, ≥22.0 respectively. Analyte, metabolite and Internal standards carry over were ≤3.92, ≤15.31, ≤0.10 & ≤0.07 respectively. Auto sampler carry over test was performed to ensure that no significant affect the precision and accuracy over the proposed method. Very negligible % carry over (≤7.00 for DES, ≤0.41 for DES-D_4 and ≤12.02 for 3-0H DES ≤0.65 for 3-OH DES-D_4) during batch run observed in extracted blank plasma after subsequent injection of highest calibration standard at the retention time of analytes.

Stability

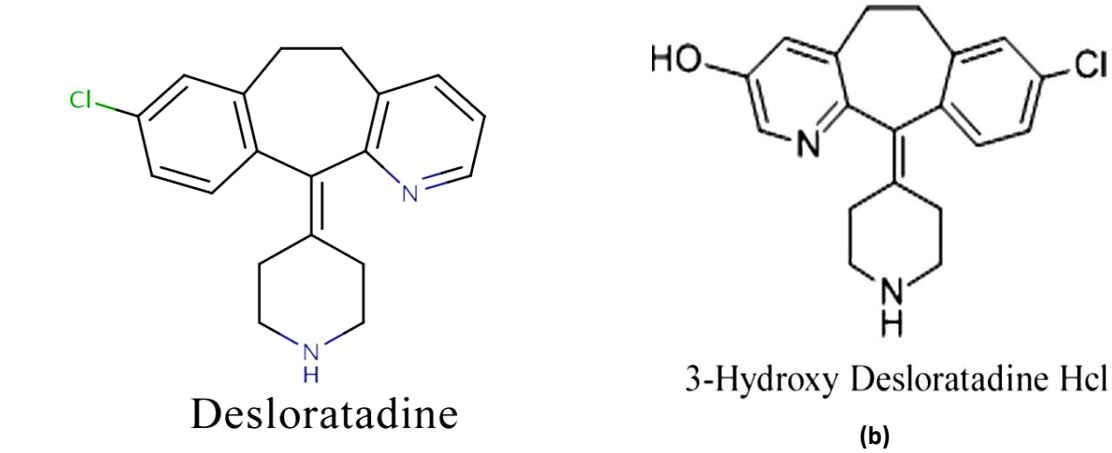
Short term stability of stock solutions reminded for 09 hours at ambient temperature for drug and ISs with 100µg/mL concentration at ULOQ and LLOQ QC level in methanol. Working solutions short term stable for 09 hours ambient temperature for both IS with 25ng/mL concentration in diluent. Long term stock solution stability of drug and metabolite stable for a minimum of167 hours at 5±3°C for 100µg/mL concentration in Methanol**.** Whereas long term stability of spiking solutions at ULOQ and LLOQ QC level were stable for 167 Hours at 5±3°C in diluent (Formic Acid in Methanol (0.1% V/V): Water, 50:50V/V). Drug stability at the LQC and HQC concentrations was measured after 05 freeze thaw cycles at -20±5°C and -78±8°C. An auto sampler Re-injection and Reproducibility at LQC and HQC concentrations for 76 Hours at 5±3°C in (Organic mixture: 10mM Ammonium Formate, (70:30V/V)) was measured and compared with freshly prepared samples. Wet extract stability for drug and metabolite were stabled for 55 Hours at 5±3°C in (Organic

mixture: 10mM Ammonium Formate, (70:30V/V)). Dry extract stability reminded for 25 Hours at-20±5°C.

CONCLUSION

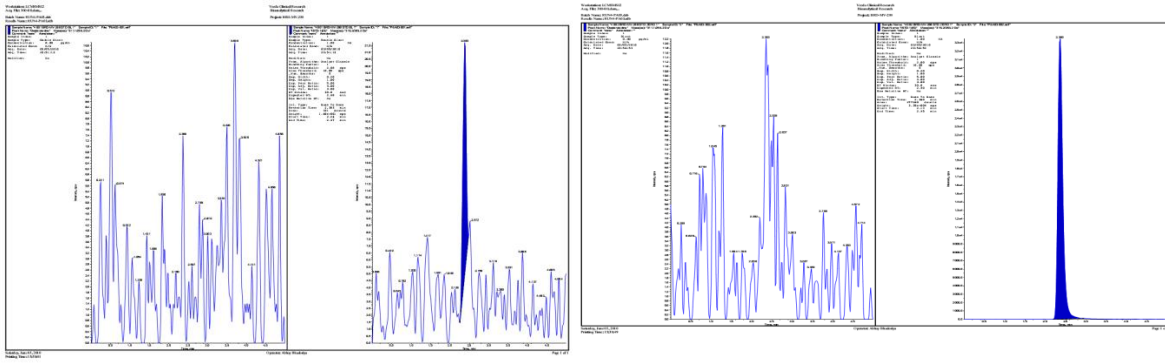
The sensitivity and selectivity of the method will enable for the simultaneous estimation of different types & strengths of desloratadine formulation in biological matrix. Being use of deuterated compounds in this method which will enables ruggedness over a period of time. An efficient extraction procedure to give reproducible and quantitative recovery at optimum level. The method is considered valid for the extraction and analysis over a linearity range 50.0pg/mL to 10000pg/mL in K_3EDTA human plasma samples. The assay was successfully applied to determine concentration of the drug and its metabolite for BABE study by following current regulatory guidelines.

Figure 1: Chemical structures of (a) Desloratadine and (b) 3-Hydroxy Desloratadine.



(a) **(b)**

Figure 2: Representative chromatograms of blank plasma and standard zero with internal standards for Drug and metabolite respectively.



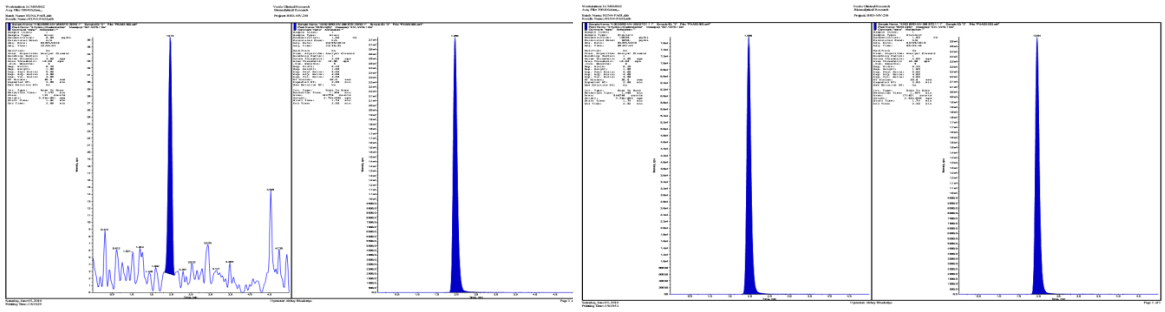


Figure 3: The representative chromatograms of a calibration standard at the LLOQ QC level.

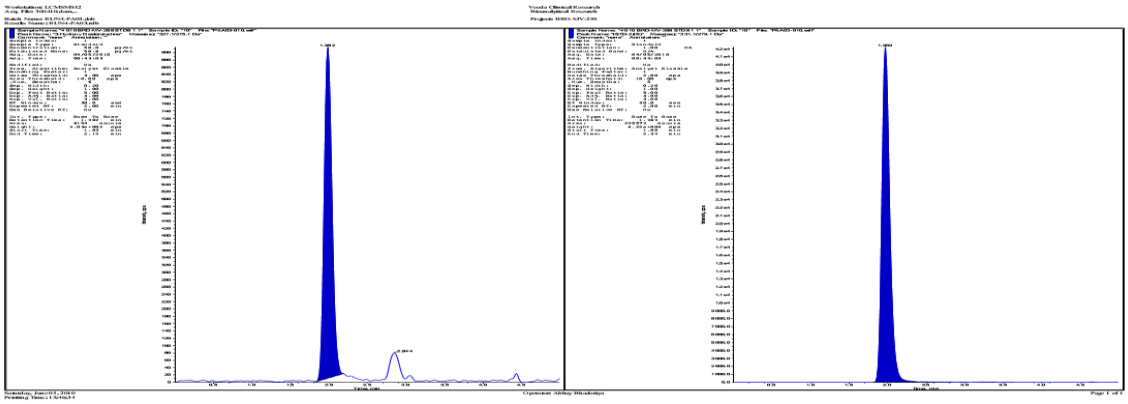


Table 1(a): Recovery percentage of Desloratadine.

Replicate No.	HQC		MQC		LQC	
	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio
1	2.975	4.585	1.527	2.325	0.052	0.083
2	2.996	4.452	1.500	2.280	0.052	0.081
3	2.998	4.508	1.516	2.310	0.051	0.081
4	2.987	4.590	1.473	2.348	0.050	0.081
5	3.001	4.603	1.479	2.306	0.050	0.082
Mean	2.9914	4.5476	1.4990	2.3138	0.0510	0.0816
SD	0.01055	0.06514	0.02318	0.02506	0.00100	0.00089
% CV	0.35	1.43	1.55	1.08	1.96	1.10
% Mean Recovery	82.2		81.0		78.1	
% Overall Recovery	80.44					
% Overall CV	2.61					

Table 1(b): Recovery percentage of 3- Hydroxy Desloratadine.

Replicate No.	HQC		MQC		LQC	
	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio	Extracted Peak Area Ratio	Un-extracted Peak Area Ratio
1	2.730	5.485	1.373	2.836	0.050	0.105
2	2.780	5.512	1.344	2.761	0.050	0.101
3	2.749	5.491	1.345	2.831	0.050	0.098
4	2.668	5.512	1.339	2.773	0.051	0.102

5	2.666	5.486	1.353	2.767	0.050	0.105
Mean	2.7186	5.4972	1.3508	2.7936	0.0502	0.1022
SD	0.05038	0.01370	0.01339	0.03671	0.00045	0.00295
% CV	1.85	0.25	0.99	1.31	0.89	2.89
% Mean Recovery	61.8		60.4		61.4	
% Overall Recovery	61.22					
% Overall CV	1.15					

Table 2: Accuracy and Precision Batch runs for Desloratadine & 3-Hydroxy Desloratadine.

Run Day No.	Curve Number	LLOQ QC (50.0 pg/mL)	% Bias	LQC (150 pg/mL)	% Bias	MQC (4000 pg/mL)	% Bias	HQC (8000 pg/mL)	% Bias
	1	48.5	-3.00	145	-3.33	4040	1.00	8130	1.63
		51.2	2.40	139	-7.33	4030	0.75	8180	2.25
Day 1		47.0	-6.00	142	-5.33	4070	1.75	8240	3.00
		49.6	-0.80	134	-10.67	4140	3.50	8220	2.75
		47.4	-5.20	142	-5.33	4050	1.25	8220	2.75
Intra run Mean		48.7		140		4070		8200	
Intra run SD		1.71		4.16		43.9		43.8	
Intra run % CV		3.51		2.97		1.08		0.53	
Intra run % Bias		-2.60		-6.67		1.75		2.50	
n		5		5		5		5	
	4	47.2	-5.60	148	-1.33	4220	5.50	8210	2.63
		48.6	-2.80	140	-6.67	4220	5.50	8540	6.75
Day 2		47.4	-5.20	144	-4.00	4200	5.00	8280	3.50
		45.2	-9.60	145	-3.33	4150	3.75	8440	5.50
		47.8	-4.40	141	-6.00	4110	2.75	8520	6.50
Intra run Mean		47.2		144		4180		8400	
Intra run SD		1.26		3.21		48.5		147	
Intra run % CV		2.67		2.23		1.16		1.75	
Intra run % Bias		-5.60		-4.00		4.50		5.00	
n		5		5		5		5	
	12	50.9	1.80	151	0.67	4030	0.75	8130	1.63
		49.8	-0.40	148	-1.33	4210	5.25	8370	4.63
Day 3		48.2	-3.60	147	-2.00	4150	3.75	8150	1.88
		50.2	0.40	148	-1.33	4120	3.00	8410	5.13
		55.9	11.80	140	-6.67	4070	1.75	8450	5.63
Intra run Mean		51.0		147		4120		8300	
Intra run SD		2.91		4.09		69.9		151	
Intra run % CV		5.71		2.78		1.70		1.82	
Intra run % Bias		2.00		-2.00		3.00		3.75	
n		5		5		5		5	
Mean Concentration Found (pg/mL)		49.0		144		4120		8300	
Inter-run SD		2.50		4.47		70.4		143	
Inter-run % CV		5.10		3.10		1.71		1.72	
Inter-run % Bias		-2.00		-4.00		3.00		3.75	
n		15		15		15		15	

Table 3(a): Ion source parameters

Curtain gas (CUR)	20.00
Ion spray voltage (IS)	5500.00
Temperature (TEM)	500.00
Nebulizer gas(GS1)	50.00
Heater gas(GS2)	60.00
Interface Heater(ihe)	ON
Collision gas (CAD)	3.00

Table 3(b): Compound dependent parameters

Parameters	Desloratadine	3- Hydroxy Desloratadine	Desloratadine D_4	3- Hydroxy Desloratadine D_4
Declustering potential (DP)	100.00	86.00	100.00	86.00
Entrance potential (EP)	10.00	10.00	10.00	10.00
Collision energy (CE)	33.00	30.00	33.00	30.00
Collision cell exit potential (CXP)	15.00	8.00	15.00	8.00
Dwell time (milli seconds)	200	200	200	200

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