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A VALIDATED ANALYTICAL METHOD FOR QUANTIFICATION OF CRISABOROLE IN MATRICES BY UPLC-ESI-MS/MS

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Keywords:

Crisaborole, Human plasma, UPLC-ESI-MS/MS, Aqueous, Bio-analysis

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ABSTRACT: The validated analytical method was applied for the estimation of Crisaborole in aqueous and human plasma with Crisaborole-D4 as an internal standard by using UPLC-ESI-MS/MS. The chromatographic separation was achieved with 10mM ammonium acetate buffer solution (pH- 4.5): Methanol, (10:90) (% v/v) using the Xterra C18, 100 × 4.6, 5µ. The total analysis time was 2 min, and the flow rate was set to 0.5 ml/min. The mass transitions of Crisaborole and Crisaborole-D4 obtained were m/z 252.1 → 222.1 and 256.1 → 222.1. The standard curve shows a correlation coefficient (r^2) greater than 0.999 with a range of 75.00-225.00 ng/ml using the linear regression model.

INTRODUCTION: Crisaborole (CB) is a novel oxaborole approved by FDA as a topical treatment of for mild to moderate atopic dermatitis. This non-steroidal agent [1-8] is efficacious in improving disease severity, reducing the risk of infection, and reducing the signs and symptoms in patients 2 years old and older. It reduces the local inflammation in the skin and prevents further exacerbation of the disease with a good safety profile. Its structure contains a boron atom, which facilitates skin penetration and binding to the bimetal center of the phosphodiesterase 4 enzyme. It is used as a topical treatment of psoriasis [9-11]. Crisaborole is (4-[(1-hydroxy-1, 3-dihydro-2, 1-benzoxaborol-5-yl) oxy]benzonitrile with chemical formula $C_{14}H_{10}BNO_3$ and its mol. weight is 251.05.

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The chemical structure of Crisaborole (CB) and its internal Standard (IS) - CBD4 was shown in **Fig. 1**.

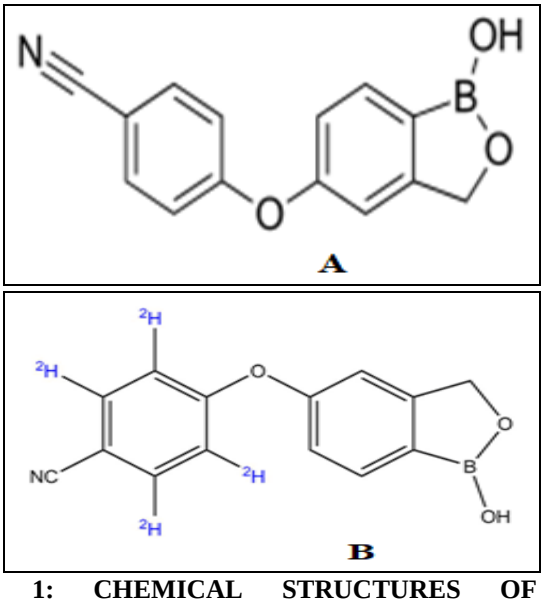

FIG. 1: CHEMICAL STRUCTURES OF A) CRISABOROLE (CB) B) CRISABOROLE-D4 (CBD_4)

The literature survey reveals that pharmacokinetic and clinical trials were reported on Crisaborole [3-9]. There is no reported hyphenated analytical method

for estimation of Crisaborole using the deuterated internal standard as CBD4 in Aqueous standard and spiked plasma samples. Hence, the main objective of the present study is to develop and validate the novel simple, sensitive, selective and reproducible analytical method for the quantitative determination of Crisaborole (CB) in standard aqueous samples by UPLC-MS/MS as per ICH analytical method validation guidelines (Q2(R1)) [13-14].

MATERIALS AND METHODS:

Materials:

Chemical Resources: Crisaborole (TLC Pharma-Chem), Crisaborole - D_4 (TLC Pharmachem), UPLC grade methanol (J. T. Baker, Phillipsburg, NJ, USA), acetic acid, ammonium acetate (S.D Fine Chemicals, Mumbai), dichloromethane (Merck Pvt. Ltd, Worli, Mumbai), ultrapure water (Milli - Q system, Millipore, Bedford, MA, USA), human plasma (Doctors Pathological Labs). The chemicals and solvents were used in this study analytical and UPLC grade.

Instrument Resources: Chromatographic separation was performed on a QSight® Triple Quad UPLC-ESI-MS/MS system (Perkin Elmer) Combined with QSight LX50 UHPLC, data acquisition and processing were accomplished using Simplicity™ 3Q software.

Microbalance (ME5 model Sartorius), variable range micropipette (Eppendorf), Autosampler vials, variable size glass bottles, graduated measuring cylinders, volumetric flasks (Borosil), Ultrasonic bath (Pharmatek Scientific), Vortexer (Spinix), Refrigerator (LG). Pipette tips 10 µL-1000 µL were employed in the present investigation.

Methods:

Preparation of 10mM Ammonium Acetate Buffer Solution: Weighed about 0.77 gm of Ammonium acetate was dissolved in 1000 mL Milli-Q water and adjust pH to 4.5 with acetic acid. Filtered through 0.45 µ-membrane disc filter and sonicated to degas.

Preparation of Mobile Phase: The mobile phase used was 10mM ammonium acetate buffer solution (pH- 4.5): methanol, (10:90) (% v/v) and the mobile phase was filtered through 0.45 µ membrane filter and sonicated before use.

Preparation of Standard Stock Solution: Standard stock solution of Crisaborole (CB) and Crisaborole-D_4 (1 mg/mL) were prepared by accurately weighing about 10 mg and transferring into 10 mL volumetric flask and dissolved in methanol. All stock solutions were stored in refrigerated conditions (2-8 °C) until analysis.

Preparation of Internal Standard Spiking Solution: The Crisaborole-D_4 (internal standard) spiking solution (100.00 ng/mL) was prepared from standard stock solution of Crisaborole-D_4 (1000.00 µg/mL) in mobile phase (10mM ammonium acetate buffer solution (pH- 4.5): methanol, (10:90) (% v/v). Internal standard spiking solution (Crisaborole-D_4) was stored in refrigerated conditions (2-8 °C) until analysis.

Preparation of Aqueous Standard Solutions: Standard solutions of different concentrations of Crisaborole (CB) were prepared from Crisaborole stock solution (1000 µg/mL) in mobile phase (10mM ammonium acetate buffer solution (pH-4.5): methanol, (10:90) (% v/v)). To each standard aqueous solution, 50 µL of 100.00 ng/mL of Crisaborole-D_4 was added and vortexed for 5 min and injected into the UPLC-ESI-MS/MS for analysis.

Method Development: For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate mass parameters and choice of stationary and mobile phase, internal standard, extraction solvent. The following studies were conducted for this purpose.

Selection of Internal Standard: For the selection of internal standard; tacrolimus, pimecrolimus, diphenhydramine, hydroxyzine were tried with optimized mobile phase and column conditions. Finally, Crisaborole-D_4 was selected as IS (internal standard) due to its compatibility with analyte chromatographic conditions. The peak elution times for the Crisaborole and Crisaborole-D_4 were found at 0.83 and 0.78 min.

Optimization of Mass spectroscopic Parameters: The pure drug of Crisaborole and Crisaborole - D_4 were prepared in methanol (100.00 ng/mL) and

injected with a flow rate of 5µL/min into positive ion mode mass spectrometer for optimization of mass parameters like source temperature, IS, heater gas, nebulizer gas, curtain gas, CAD gas (all gas channels were purged with ultra-high pure nitrogen gas), EP, DP, CE, FP, and CXP were optimized. The analysis was performed using MRM positive ion mode with mass transitions of m/z (amu) 252.1→222.1 and 256.1→222.1 for Crisaborole and Crisaborole-D_4. The mass spectras of parent and product ions were depicted in **Fig. 2**.

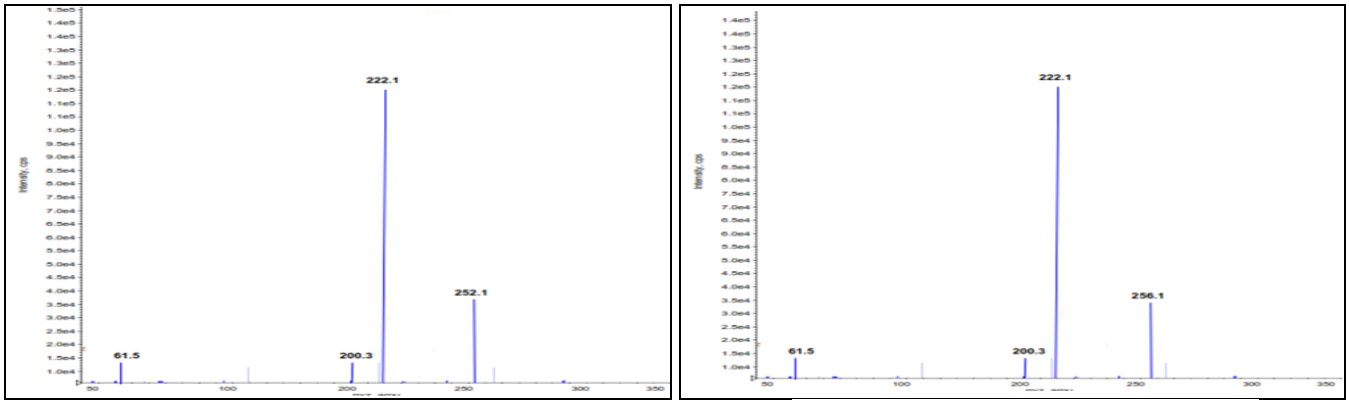

FIG. 2: PARENT ION MASS SPECTRA (Q1) AND (Q3) OF CRISABOROLE & CRISABOROLE-D_4

Optimized Chromatographic Conditions: Several systematic trials were performed by varying stationary phases and mobile phase ratios to achieve ideal chromatographic conditions. The chromatographic separation was achieved with 10mM ammonium acetate buffer solution (pH- 4.5) in combination with methanol (10:90) (% v/v) using the Xterra $C_{18,}$ 100×4.6, 5µ gave the best peak shape, and low baseline noise was observed. The total analysis time was 2 min, and the flow rate was set to 0.5 ml/min. The temperature was set to 40°C for the column oven. The sample volume for the injection into mass spectrometry was adjusted to 10 µl for better ionization and chromatography.

Bioanalysis of Crisaborole in Plasma Spiked Samples:

Development of Extraction Solvent: Various organic solvents were optimized to extract Crisaborole and Crisaborole-D_4 from the spiked plasma samples. After a series of trials, dichloromethane was selected as appropriate due to high recovery efficiency and matrix-free interference.

Sample Extraction and Cleanup Procedure (Sample Preparation): To each labeled polypropylene tube 50 µL of IS (100.00 ng/mL) was mixed with the 100 µL screened plasma spiked sample, and 2.5 mL of dichloromethane were added, vortexed for 5 min and centrifuged at 5000 rpm for 10 min at 20°C. The organic phase was transferred to a clean polypropylene tube and dried with nitrogen gas at 40°C. The residue was reconstituted with 200 µL mobile phase and injected into the UPLC-ESI-MS/MS for analysis. After completion of several systematic trials, a sensitive, precise and accurate UPLC-MS/MS method was developed for the analysis of Crisaborole (CB) and Crisaborole -D_4 in Aqueous standard solutions and spiked plasma samples. The chromatograms of blank (Mobile phase), Blank (Plasma), Aqueous standards (LOQ and ULOQ), and plasma spiked standard sample were shown in **Fig. 2-7**. This was followed by method validation.

Method Validation: The developed method was validated over a linear concentration range of 75.0–225.0 ng/ml. The validation parameters include selectivity and specificity, LOQ, linearity, precision and accuracy, robustness, recovery, stability in solution, and plasma was evaluated under the validation section.

System Suitability: Six replicate injections of aqueous standard 100% level (150.0 ng/mL) along with internal standard (100.0 ng/mL) were injected into UPLC-MS/MS and % RSD was calculated.

Selectivity & Specificity: The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

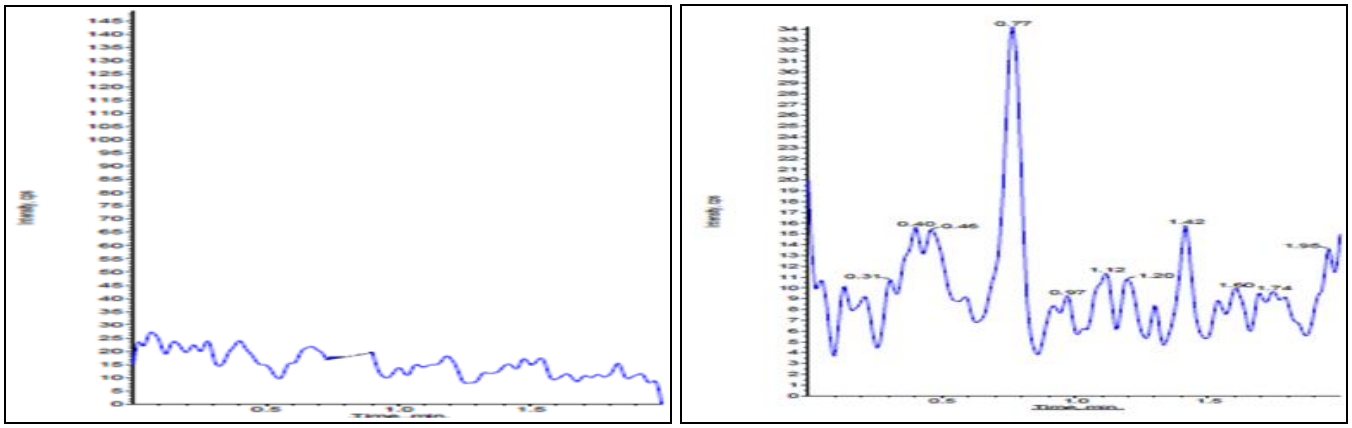

FIG. 3: BLANK CHROMATOGRAM (MOBILE PHASE)

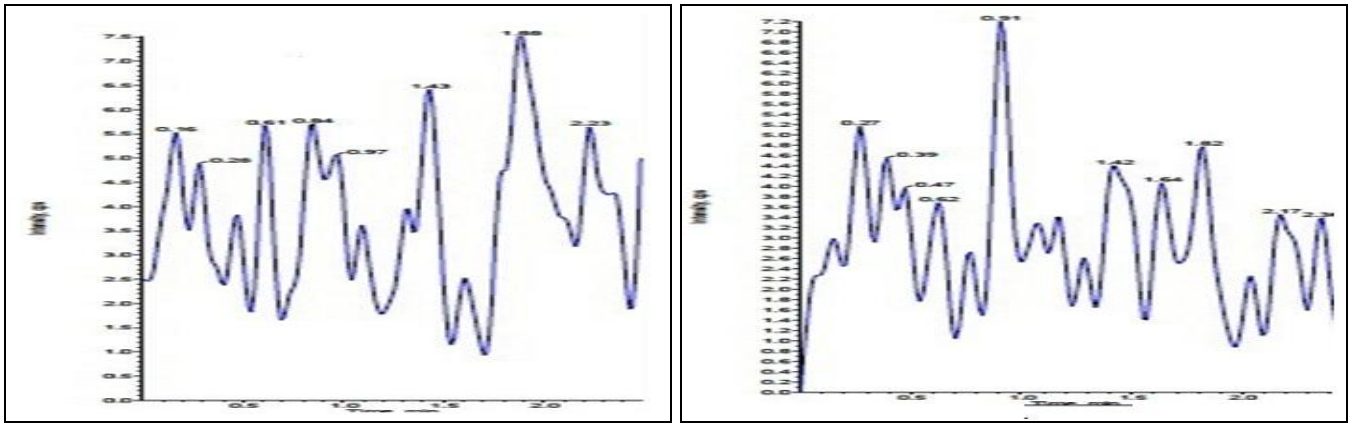

FIG. 4: BLANK CHROMATOGRAM (PLASMA)

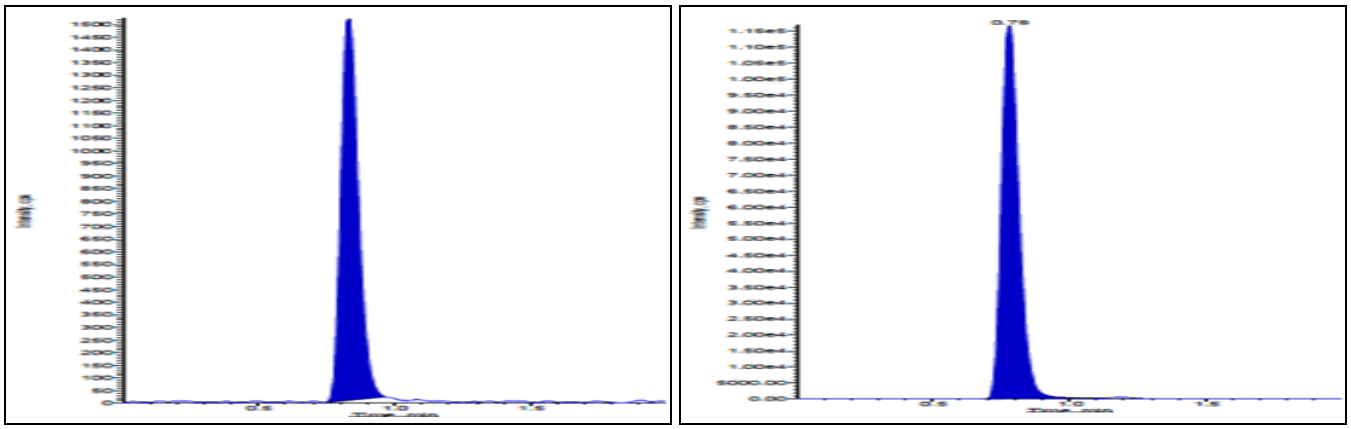

FIG. 5: STANDARD CHROMATOGRAM OF LOQ SAMPLE (50% LINEARITY LEVEL) (CB AND CB-D_4)

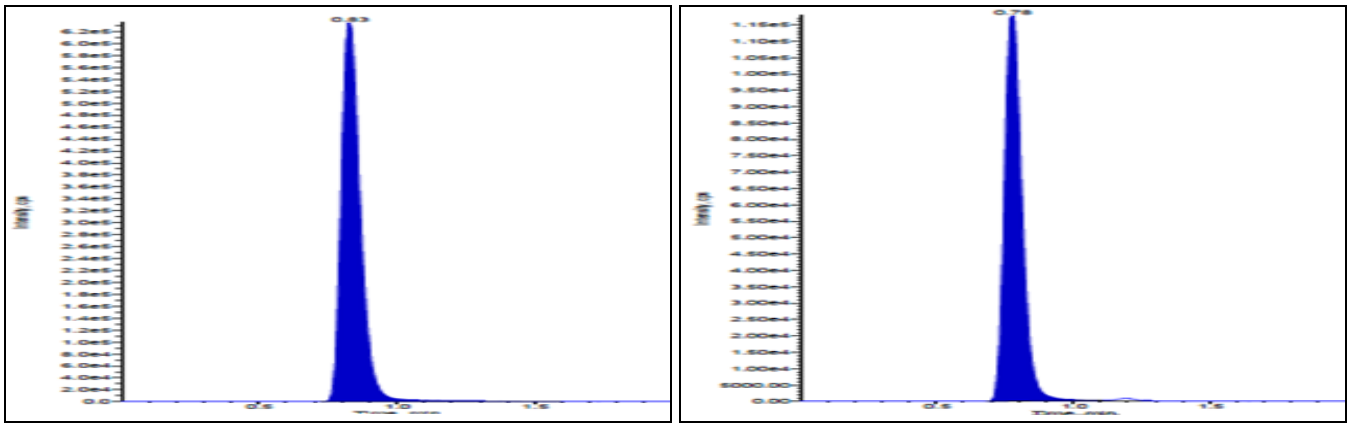

FIG. 6: STANDARD CHROMATOGRAM OF ULOQ SAMPLE (150% LINEARITY LEVEL) (CB AND CB-D_4)

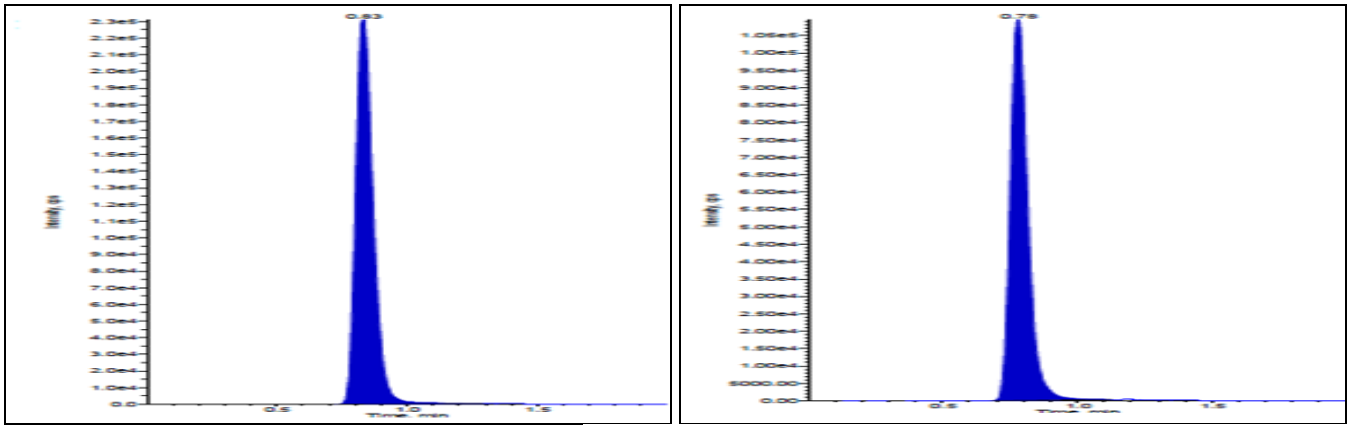

FIG. 7: STANDARD CHROMATOGRAM OF CRISABOROLE & CRISABOROLE-D_4 IN SPIKED PLASMA SAMPLES

If an analytical procedure can separate and resolve the various components of a mixture and detect the analyte qualitatively, the method is called selective. It has been observed that there were no peaks of diluents and placebo at the main peaks. To test the interference at the retention time of Crisaborole (CB) and Crisaborole-D_4 (CBD4), blank samples (Mobile phase and Plasma) were injected into UPLC-MS/MS. Hence, the chromatographic system used for the estimation of Crisaborole (CB) and Crisaborole-D_4 were very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 0.83 min for Crisaborole (CB) and 0.78 min for Crisaborole -D_4 (IS).

Linearity and Range: The linearity of the calibration curve for Crisaborole was assessed at 50 % to 150 % of the target concentration at different levels in the range of 75.0 ng/mL to 225.0 ng/mL in aqueous standards.

TABLE 1: CALIBRATION CURVE DETAILS OF CRISABOROLE (CB)

Linearity Level (%)	Nominal Conc. (ng/mL)	Crisaborole	Crisaborole-D4	Mean Peak Area Ratio (n=3)
		Mean Peak Area (n=3)	Mean Peak Area (n=3)	
50	75.00	261353	63742631	0.0041
60	90.00	313623	62612610	0.0050
70	105.00	365894	62482629	0.0059
80	120.00	418164	62352628	0.0067
90	135.00	470435	62222627	0.0076
100	150.00	522705	62992626	0.0083
110	165.00	574976	62762625	0.0092
120	180.00	627246	63732624	0.0098
130	195.00	679517	62702603	0.0108
140	210.00	731787	63772622	0.0115
150	225.00	784058	63742621	0.0123
		Correlation coefficient		0.9995
		Y-Intercept		0.000067

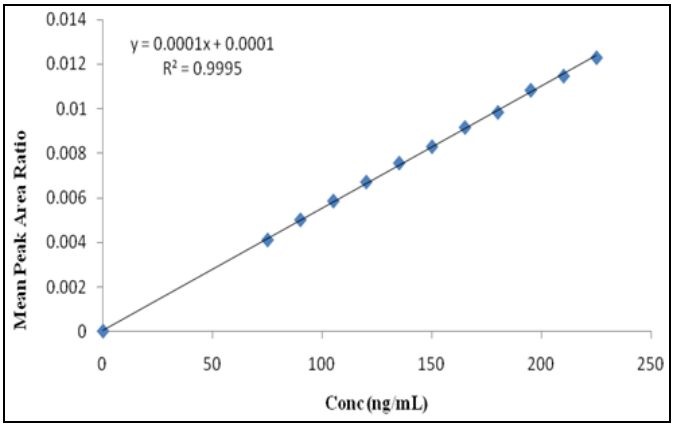

FIG. 8: CALIBRATION CURVE FOR CRISABOROLE (CB)

Peak area ratios for each solution against its corresponding concentration were measured, and the calibration curve was obtained from the least-squares linear regression presented with their correlation coefficient.

Precision: The intra-day data reflects the precision and accuracy of the method under the same conditions within one day. Intra-day accuracy and precision were obtained by analyzing ten replicates of three different standard samples (120, 150, and 180 ng/mL).

Accuracy was determined by the regressed (measured) concentration represented as a percentage of the target (nominal) concentration. The percent relative standard deviation (% RSD) of the regressed (measured) concentrations were used to report precision. The inter-day precision and accuracy were verified by repeating the above procedure at three different days.

TABLE 2: PRECISION AND ACCURACY OF CRISABOROLE AT THREE DIFFERENT CONCENTRATIONS

Concentration (ng/ml)	Within-run (Intra-day)			Between-run (Inter-Day)		
	Mean Concentration measured (n=10;ng/ml; mean ± S.D)	% CV	% Accuracy	Mean Concentration measured (n=30;ng/ml; mean ± S.D)	% CV	% Accuracy
120.00	120.92±1.38	1.15	100.77	122.13±2.08	1.70	101.78
150.00	150.05±1.31	0.87	100.03	149.46±1.54	1.03	99.64
180.00	179.39±2.64	1.47	99.66	179.78±1.30	0.72	99.88

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection (LOD) and limit of quantification (LOQ) of Crisaborole was determined by calibration curve method. Solutions of Crisaborole were prepared in linearity range and injected in triplicate. The average peak area ratio of the three analyses was plotted against concentration. LOD and LOQ were calculated by using the following equations:

$$LOD = 3.3 \times \sigma / S \text{ and } LOQ = 10 \times \sigma / S$$

Where σ is the standard deviation of y-intercepts of regression lines, and S is the slope of the calibration curve.

Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature and determine the effect (if any) on the results of the method. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. Robustness was carried by carried out by varying the method parameters like flow rate (± 5%), column temperature (± 5%), and pH (± 2%). Six replicate injections of aqueous standard 100% level (150.0 ng/mL) along with internal standard was injected to UPLC-ESI-MS/MS and % RSD was calculated.

TABLE 3: ROBUSTNESS OF CRISABOROLE

Validation Sample	% RSD		
	Flow Rate (± 5%)	Column Temp. (± 5%)	pH High (± 2%)
Crisaborole (150.00ng/mL)	0.51	0.33	0.91

Stability: Stability of Crisaborole and Crisaborole-D_4 in aqueous standards was performed using two replicates of 150.0 ng/mL and 100.00 ng/mL at ambient and refrigerated conditions with different time intervals.

Solution Stability: A Crisaborole (CB) at concentration of 150 ng/mL solution and Crisaborole-D_4 (IS) solution at 100.0ng/mL of were prepared from fresh stock solutions. A portion of the freshly prepared standard solutions (Crisaborole and Crisaborole-D4) were kept at ambient temperature (25 °C) for 36 h and then analyzed by the proposed method. A second portion of the freshly prepared standard solutions (Crisaborole and Crisaborole-D4) were stored at refrigerated temperature (between 2°C and 8°C) for 36 h and then analyzed. The results were compared with those obtained from samples analyzed at the initial moment (0.0 h).

TABLE 4: SOLUTION STABILITY DATA OF CRISABOROLE AND CRISABOROLE-D_4

Stability Sample	Ambient Temperature		Refrigerated Temperature	
	% Difference at 0.0 h	% Difference at 36.0 h	% Difference at 0.0 h	% Difference at 36.0 h
Crisaborole (150.00ng/mL)	0.0001	0.0264	-0.4616	0.9266
Crisaborole –D_4 (100.00ng/mL)	0.0001	-1.2981	0.2016	-0.7915

Filter Validation (Filter Interference): A Crisaborole (CB) at a concentration of 150.00ng/mL solution and Crisaborole -D_4 (IS) solution at 100.00 ng/mL of were prepared from fresh stock solutions. Some portion of Crisaborole (CB) and Crisaborole-D4 (CBD4) standard

solutions (150.00ng/mL and 100.00ng/mL) was filtered through three different filters namely 0.45µm PVDF filter, 0.45µm PTFE and 0.45µm nylon filter and some portion was centrifuged and injected into the UPLC- ESI-MS/MS system.

TABLE 5: FILTER INTERFERENCE RESULTS OF CRISABOROLE AND CRISABOROLE-D_4

Validation Sample	%Difference		
	0.45µm Nylon	0.45µm PVDF	0.45µm PTFE
Crisaborole (150.00ng/mL)	0.42	0.21	0.51
Crisaborole–D_4 (100.00ng/mL)	0.89	0.52	0.99

Bioanalysis of Crisaborole in Plasma Spiked Samples:

Sample Extraction and Cleanup Procedure (Sample Preparation): To each labeled polypropylene tube 50 µL of IS (100.00 ng/mL) was mixed with the 100 µL screened plasma spiked sample and 2.5 mL of dichloromethane were added, vortexed for 5 min and centrifuged at 5000 rpm for 10 min at 20 °C. The organic phase was transferred to a clean polypropylene tube and dried with nitrogen gas at 40 °C. The residue was reconstituted with 200 µL mobile phase and injected into the UPLC-ESI-MS/MS for analysis.

Extraction Efficiency (Recovery): The extraction recovery of analyte (Crisaborole) at three (75, 150, 225 ng/mL) different concentrations were determined by measuring the peak area responses from screened plasma samples spiked with Crisaborole (Extracted samples) with those Aqueous standard samples (Un-extracted samples). The samples were extracted as per the sample extraction and cleanup method.

The recovery of IS (Crisaborole-D4) at a concentration of 100.00 ng/mL was determined in the same way. The recovery of Crisaborole and Crisaborole-D_4 were determined using six replicates. The % recovery (Extraction recovery) at three concentration levels was obtained according to the following equations. The results were depicted in **Table 6**.

% Recovery = Mean extracted drug peak area / Mean un-extracted drug peak area × 100

Mean % Recovery = Mean extracted % Recovery / Mean un-extracted drug peak area × 100

TABLE 6: RECOVERY OF CRISABOROLE AND CRISABOROLE-D_4 IN SPIKED PLASMA SAMPLES

Concentration (ng/ml)	Crisaborole				Crisaborole-D_4			
	% Recovery	% CV	Mean % Recovery (n=18)	Mean % CV (n=18)	% Recovery	% CV	Mean % Recovery (n=18)	Mean % CV (n=18)
75.00	98.09	0.71	98.93	0.64	98.54	0.84	99.73	1.02
150.00	98.18	0.56			101.43	0.89		
225.00	100.51	0.65			99.21	1.32		

Long-Term Stability (-30°C, 30 Days): Drug (Crisaborole**)** spiked plasma samples with a concentration of 150.00 ng/mL (LT Stability samples) of six replicates were stored at -30 °C freezer up to 30 days.

TABLE 7: LONG-TERM STABILITY (-30°C, 30 DAYS) OF CRISABOROLE IN PLASMA SAMPLES

Stability sample	% CV	
	Comparison samples (0 days)	LT Stability samples (30 days)
Crisaborole (150.00 ng/mL)	1.07	0.95

After completion of the stability period stability samples were removed from -30 °C freezer and processed as per samples extraction procedure. Stability of Crisaborole in plasma samples (at -30 °C up to 30 days) were assessed by comparing with freshly prepared drug (Crisaborole) spiked plasma samples (Comparison samples) with the concentration of 150.00 ng/mL (LT Comparison samples) of six replicates.

RESULTS AND DISCUSSION:

Method Development: The main aim of this work was to develop a rapid, selective and sensitive analytical method including an efficient and reproducible sample clean-up step for quantitative analysis of Crisaborole in aqueous and plasma spiked standard samples. Optimization of analysis in plasma, dichloromethane was added to plasma samples to increase extraction efficiency, resulting in higher extraction efficiency. Subsequently, a simple and inexpensive extraction procedure that could be implemented in monitoring laboratories provided an assay well suited for real-time analyses. In optimizing the chromatographic conditions, the ammonium acetate buffer solution

was adopted in the mobile phase of the UPLC to suppress the tailing phenomena of chromatographic peaks of Crisaborole and Crisaborole-D_4. Besides, the concentration of the ammonium acetate buffer was investigated, and the concentration of 10mM ammonium acetate (pH: 4.5) made the chromatographic peaks sharp and symmetric. The acceptable retention and separation of Crisaborole and Crisaborole-D4 were obtained by using an elution system of 10mM ammonium acetate (pH: 4.5): methanol (10:90, v/v) as the mobile phase.

The UPLC/MS/MS method described here satisfies the requirement of routine analyses since it has a short run time (2 min). The MS optimization was performed by direct injection of Crisaborole and Crisaborole-D_4 into the mass spectrometer. The mass parameters were optimized to obtain better ionization of Crisaborole and Crisaborole-D_4 molecules. The full scan spectrum was dominated by protonated molecules [M+H] m/z 252.1 and 256.1 for Crisaborole and Crisaborole-D_4, and the major fragment ions observed in each product spectrum were at m/z 222.1 and 222.1, respectively.

Method Validation:

System Suitability: System suitability parameter can be defined as a test to ensure that the method can generate precise results. In this method % RSD value obtained was less than 2%.

Selectivity and Specificity: No significant response was observed at retention times of Crisaborole and Crisaborole-D_4 in the mobile phase and blank plasma samples. It can be concluded that the method is specific for estimation of Crisaborole in the presence of matrices. The chromatograms of blank samples (Mobile phase and Plasma) Crisaborole standards (Aqueous and plasma spiked) were shown in **Fig. 2-7**.

Linearity: Linearity was plotted as a peak area ratio (Crisaborole peak area / Crisaborole-D_4 peak area) on the y-axis against Crisaborole concentration (ng/ml) on the x-axis. The correlation coefficient for Crisaborole over the concentration range of 75.0 to 225.0 ng/mL was 0.9995 **Table 1** and **Fig. 8**. The regression equation for Crisaborole was, y = 0.0001x + 0.0001. Linearity was found to be quite satisfactory and reproducible.

Precision & Accuracy: The precision of the proposed method was evaluated at three different concentration levels, and Accuracy and % RSD for each concentration values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on different days. No statistically significant difference was observed. The % RSD and accuracy was found to be 0.87 to 1.47 and 99.66 to 100.77% for intraday precision. Whereas, for interday precision % RSD and accuracy were found to be 0.72 to 1.70 and 99.64 to 101.78%. The result was summarized in **Table 2**.

LOD and LOQ: The detection and quantification limits were evaluated from calibration curve plotted in the concentration range of 75.0-225.0 ng/mL. LOD and LOQ for this method were found to be 4.30 and 14.32 ng/mL, respectively. These values indicated that the method was sensitive to quantify the drug.

Robustness: The % RSD of Crisaborole was good under most conditions and didn't show any significant change when the critical parameters were modified and the components (Analyte and IS) were well separated under all the changes carried out **Table 3**. Thus the method conditions were robust.

Solution Stability: The processing and storage conditions of samples need to maintain the integrity of a drug or at least keep the variation of pre-analysis as minimal as possible. For this reason, stability studies play an important role in analytical method development. In this study, the stability was assessed by considering stock solution stability for Crisaborole and Crisaborole-D_4 **Table 4** show that stable under the studied conditions (Ambient and Refrigerated conditions). Since in all conditions, the % difference values were smaller than 2%.

Filter Validation (Filter Interference): Peak Areas of filtered standard solutions were compared against unfiltered standard (Centrifuged) solution. The % Difference values for Crisaborole and Crisaborole-D_4 of different filter materials was found to be 0.21 to 0.51% and 0.52 to 0.99 **Table 5**. No significant interference was observed. Hence, 0.45 µm size of Nylon, PTFE, and PVDF filters were suitable for filtering of standard samples.

Bioanalysis of Crisaborole in Plasma Spiked Samples:

Recovery: The mean % recovery for three different concentrations samples of Crisaborole was 98.09%, 98.18%, and 100.51% respectively. The overall mean % recovery and % CV of Crisaborole across three concentration levels is 98.93% and 0.64%. For the Crisaborole-D_4 (internal standard), the mean % recovery and % CV is 99.73% and 1.02 **Table 6**.

Long-Term (LT) Stability (-30 °C, 30 Days): Long-term stability of an analyte (Crisaborole) was studied at −30 °C for 30 days with a % RSD was found to be 0.95%. The data are given in **Table 7**.

CONCLUSION: An alternative UPLC-ESI-MS/ MS method for quantification of Crisaborole in aqueous and human plasma has been successfully developed and validated.

A simple and inexpensive liquid-liquid extraction procedure and an isocratic chromatography condition using a reversed phase column provided an assay well suited for real-time analyses. The method exhibited excellent performance in terms of selectivity, linearity, accuracy, precision, recovery, robust, stability in various matrices. The method is sensitive enough for quantitative detection of the analyte in pharmaceutical preparations. Also, the reported method has a short analysis run time and deuterated internal standard, an advantage over previously reported methods.

The proposed method can thus be used for routine analysis, quality control, stability studies and suitable for therapeutic drug monitoring (pharmacokinetic or bioequivalence studies) of pharmaceutical tablets containing Crisaborole.

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