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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CRISABOROLE IN TOPICAL DOSAGE FORM

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Abstract

A simple, specific, accurate, precise , rapid, robust and selective stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for assay and related substances and validated for quantification of Crisaborole with its excipients in its topical dosage form. Forced degradation study is done to determine stability of the product. In mobile phase Water: Methanol (30: 70 v/v), the maximum of Crisaborole was measured to be 241nm. The technique is highly sensitive, with linearity ranging from 5 to 25g/ml (regression equation: y = 316606x - 427583; r2 = 0.9992). For verifying and testing this approach, several parameters according to ICH recommendations and USP are used. The detection and quantitation limits were determined to be 0.1094 and 0.3316 g ml-1, respectively. The results revealed that the process is accurate, specific, and repeatable (RSD 2%), as well as easy, inexpensive, and less time consuming, and suitable for determining Crisaborole in topical dosage form. The method have been robust under various variation with flow rate, detection wavelength and column oven temperature. The drug was exposed to stress conditions. Method resolves all degraded product as compared to Crisaborole. Developed method can be used routinely for estimation of drug Crisaborole with its excipient methyl paraben in dosage form and stability sample.

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Introduction

Crisaborole is a novel oxaborole endorsed by FDA on December 14, 2016 as Eucrisa, a skin treatment of for mellow to direct atopic dermatitis. Chemically, Crisaborole is 4-[(1-hydroxy-1, 3-dihydro-2, 1-benzoxaborol-5-yl)oxy]benzonitrile and having molecular formula $C_{14}H_{10}BNO_3$. The chemical structure of Crisaborole is given in fig 1.

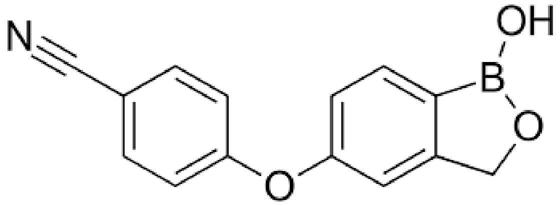

Fig.1:Structure of Crisaborole

The molecular mass of Crisaborole is 251.05 g/mol. In adults 2 years of age and older, these nonsteroidal anti-inflammatory medications may improve disease severity, lower the risk of infection, and minimize signs and symptoms. With adequate protection, you can heal the nearby skin and keep the condition from worsening. Boron atoms in this chemical improve skin penetration and are trustworthy for bimetallic contact sites of phosphodiesterase-4 stimulants. This medication is currently being developed as a psoriasis skin therapy. Scientific strategies were computed at VitalInsight employing RP-HPLC procedure, according to a published report. Liquid, on the other hand, lacked a reasonable and cautious strategy. Strategy for Chromatography As a result, the goal of this research is to create a highly sensitive and easy RP-HPLC technique for assessing Crisaborole in complex structures [1, 2].

Experimental

Instruments

The chromatographic partition was performed on Analytical Technologies HPLC-3000 arrangement smaller fluid chromatographic framework coordinated with a variable frequency programmable UV identifier and a Rheodyne injector outfitted with 20µl fixed circle. An opposite stage C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized. Model - UV 2012 twofold shaft UV obvious spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric judgments and gauging purposes individually.

Reagents and Chemicals

Crisaborole (99.88%) was obtained as gift sample from Visa Chem Private Ltd. and used as Reference Standard. Crisaborole ointment, Eucrisa (2% w/w) (Manufacturer - Pfizer Inc.) was procured from local pharmacy. HPLC grade Methanol and water were acquired from Merck specialities private restricted, Mumbai. Milli-Q water was used for the study supplied by Milli Q Plus purification system.

Chromatographic conditions

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized for the chromatographic separation at a discovery frequency of 275 nm. Water: Methanol (30: 70 v/v) was chosen as mobile phase for elution and same blend was utilized in the arrangement of standard and sample solutions. The elution was checked by infusing the 20µl and the stream rate was changed in accordance with 1.0 ml/min [3].

Preparation of standard solutions

10mg Crisaborole was precisely gauged and moved into 10 ml volumetric cups, broken up utilizing portable stage and the volume was made up with a similar dissolvable to acquire essential stock arrangement of focus 1000µg/ml of the medication (Working stock arrangement) [4].

Optimisation of RP-HPLC method

The HPLC technique was streamlined with an expect to build up an assessment of Crisaborole. Different mobile phases were gone after for the method optimisation, however satisfactory retention times, hypothetical plates and good resolution were seen with Water: Methanol (30: 70 v/v) utilizing C18 column [5] [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] (Table 1) and a run of the chromatograph of Crisaborole was appeared in figure 2.

Table 1: Optimized parameter

Parameter	Condition
Column	CosmosilC18 (250mmx 4.6ID,Particlesize: 5 micron)
Mobile Phase	Water: Methanol (30: 70 v/v)
FlowRate	1.0ml/min
Wavelength	296 nm
InjectionVolume	10 µl
Detector	UV-3000-M
RetentionTime	11.4 min (Average)

Validation of RP-HPLC method

Validation of the optimized HPLC method was performed in accordance to the ICH Q2 (R) guidelines.

Linearity

For the determination of linearity, appropriate sample solutions were pipetted out from 1000µg/ml stock solution. 0.05 – 0.25 ml was pipetted out in to five of 10ml volumetric flasks respectively and volume was made with the mobile phase to obtain concentration ranging from 5-25µg/ml of crisaborole. Each solution from flask was injected in triplicate in system. Calibration curves were plotted with concentration of solutions against observed peak areas made by them followed by the determination of regression factor and calculation of the correlation coefficients [6]. The calibration curves of Crisaborole sample was shown in figure 3.

Accuracy

To make sure the reliability and accuracy of the recovery study data were carried out by % recovery method which is also called as standard addition method [7]. A known quantity of pure drug of crisaborole was mixed to pre-analysed sample and contents again undergo analysis by the optimized method and the % recovery was reported in table 2.

Precision

The repeatability study of the proposed method was verified by calculating the percentage RSD of three replica injections of 100% concentration i.e. 6µg/ml of Crisaborole on the same day and for intraday precision % RSD was calculated from repetition [8]. The results were shown in table 3.

Limit of Quantitation (LOQ) & Limit of Detection(LOD)

The LOD and LOQ were analysed from the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formula LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was calculated by changing the chromatographic conditions like compositions of mobile phase, detection wave length, flow rate etc. and the % RSD should be reported. In the optimized conditions Small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wave length and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified n changes in the optimised conditions were injected to the system in triplicate [9].

System suitability

It was make sure that from the system suitability parameters, the method can gives results of accuracy and precision**.** System suitability was performed with three replicate injections of solution of 15 µl/ml of Crisaborole in to the chromatographic system [10].

Results and Discussion

Linearity

It was clarify from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study gave linear relationship over the concentration range of 0.05 - 0.25 µg/ml for Crisaborole. From the regression analysis, a linear equation was obtained y = 316606x - 427583, and the goodness-of-fit (r^2) was found to be 0.9992, indicating a linear relationship between the concentration of analyte and area under the peak.

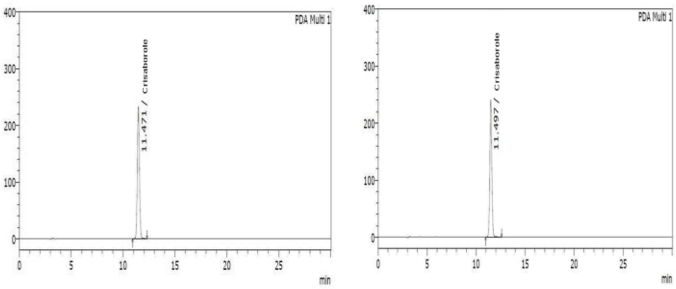

Fig. 2. Chromatogram of Crisaborole-Standard & Sample

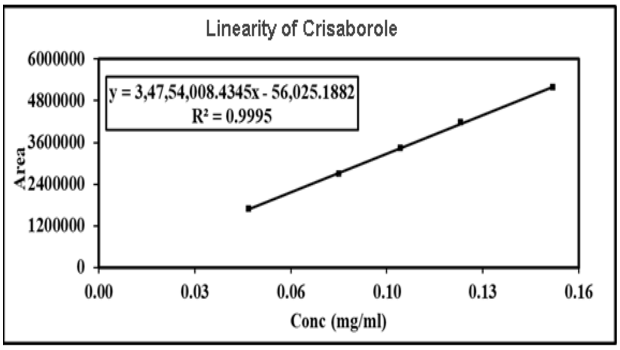

Fig 3. Linearity graph of Crisaborole – Assay

Accuracy

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Crisaborole and all the results are within limits. Acceptable accuracy was within the range and not more than 1.0% RSD, as demonstrated in Table -2.

Table 2: Summary of results of accuracy.

Level	Amount Added (mg/ml)	Amount Recovered (mg/ml)	Avg.Area	% Recovery	Mean	SD	%RSD
50%-1	0.050340	0.050375	1628097	100.07	100.78	0.64	0.63
50%-2	0.049740	0.050391	1628607	101.31			
50%-3	0.050040	0.050521	1632811	100.96			
100%-1	0.100380	0.100755	3256343	100.37	100.15	0.83	0.83
100%-2	0.099580	0.100425	3245672	100.85			
100%-3	0.100980	0.100198	3238333	99.23			
150%-1	0.151220	0.150689	4870145	99.65	100.51	0.86	0.85
150%-2	0.150420	0.151208	4886921	100.52			
150%-3	0.149120	0.151155	4885214	101.36			

Precision

Precision is "the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is expressed in the form of relative standard deviation. The repeatability, intra-day and inter-day precision results are shown in the table 3. The RSD were calculated for all the results are within limits. Precision was not more than 5.0% RSD, as demonstrated in Table 3.

Table 3 Statistical Data for Method Precision–Assay and Related Substances

Sample	Level	% Assay	% Individual impurity NMT 1%	%Total impurity NMT 2%
1	100%	101.00	0.16	0.16
2	100%	101.00	0.18	0.18
3	100%	101.00	0.17	0.17
4	100%	101.00	0.17	0.17
5	100%	101.00	0.17	0.17
6	100%	102.00	0.17	0.17
Average		**101.17**	**0.17**	**0.17**
%RSD		**0.40**	**3.72**	**3.72**

LOD and LOQ

The LOD and LOQ were calculated by the equations

$$\text{LOD} = \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$$

$$\text{and LOQ} = \frac{10 \times \text{std.Deviation}}{\text{slope}}$$

where, std.Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Crisaborole were 0.010 and 0.028μg/ml respectively.

Forced Degradation Studies

Degradation Condition	RRT	% Degradation	% Total Degradation	Peak Purity NLT 0.995	% Assay
Unstressed Sample	No Degradation Peak			1.0000	100.54
Acid Degradation(0.1 NHCl)	0.74	0.06	0.17	1.0000	99.06
	0.88	0.11			
Acid Degradation(1 N HCl)	0.89	0.06	0.06	1.0000	100.04
Alkali Degradation(0.1 NNaOH)	0.73	0.28	0.28	1.0000	99.37
Alkali Degradation(1 N NaOH)	0.73	1.23	2.07	1.0000	98.07
	0.88	0.84			
Oxidative Degradation(10% H2O2)	0.74	0.11	0.69	1.0000	99.19
	0.79	0.15			
	0.89	0.43			
Oxidative Degradation(30% H2O2)	0.20	0.12	1.92	1.0000	98.08
	0.51	0.27			
	0.58	0.35			
	0.73	0.44			
	0.79	0.21			
	0.89	0.53			
Photo Degradation	0.73	0.06	0.14	1.0000	99.27
	0.88	0.08			
Thermal Degradation (40°C)	0.73	0.07	0.07	1.0000	99.57
Thermal Degradation (60°C)	0.73	0.33	0.33	1.0000	99.13
Thermal/Humidity (25°C/60% RH)	No Degradation Peak			1.0000	99.49
Thermal/Humidity (25°C/90% RH)	0.73	0.06	0.06	1.0000	99.05

Conclusion

A simple, rapid stability indicating RP-HPLC method was developed for the determination of Crisaborole in topical dosage form. The proposed method was validated according to ICH guidelines and was reported to be accurate, precise, robust, rugged and specific. The percentage degradation was within the limit for commercial topical dosage form and the degraded peaks do not interfere with significant main peak. As there are no compendia methods available, the proposed method can be used for quality control and stability study analysis of pharmaceutical preparations. The identification of unknown impurity peaks may be taken up as further research in this study.

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Conflict of Interest

No conflict of interest.

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