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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CRISABOROLE BY RP-HPLCMETHOD IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT

A new, sensitive, suitable, clear, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of Crisoborole in bulk drug and liquid formulation was developed and validated in this research. The separation was performed using an HPLC method with a UV detector and Openlab EZchrome workstation programme, Kromasil C18**,** 250 mm X 4.6mm ID, 5 µm column, Methanol:0.1%OPAinwater (75:25%V/V)was pumpe data flow rate of1.0mL/min and detected at 307 nm.

Result-The developed RP-HPLC method yielded a suitable retention time for Crisoborole of 2.73 min, which was optimized using trial and error basis. The linearity of the established method was verified with a correlation coefficient(r^2) of 0.99999 over the concentration range of 2.0-30.0µg/mL.ThepercentageRSDforthemethod'sprecisionwasfoundtobelessthan 2.0percent.The percentage recoveries were discovered with in the limit.**0.180µg/mL** and

0.545µg/mL were found to be the LOD and LOQ, respectively.

Conclusion- The developed and validated RP-HPLC system takes less time and can be used in the industry for routine quality control/analysis of bulk drug and marketed Crisoborole products.

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Introduction:

Crisaborole is a novel oxaborole approved by FDA on December 14, 2016 as Eucrisa, a topical treatment of for mild to moderate atopic dermatitis. This non-steroidal agent 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 currently under development as topical treatment of psoriasis.

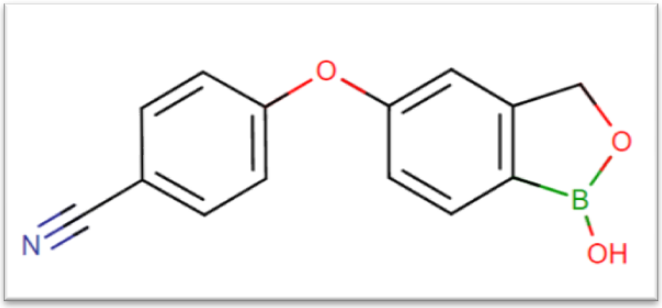

Fig-01 Structure of Crisaborole[01]

DRUG PROFILE:

Table-01 Drug Profile[02]

Category	Agents for Dermatitis, Excluding Corticosteroids
Chemical Name	4-[(1-hydroxy-1,3-dihydro-2,1-benzoxaborol-5-yl)oxy]benzonitrile
Molecular Formula	$C_{14}H_{10}BNO_3$
Molecular Weight	251.05 g/mol
Solubility	Water Insoluble

Mechanism of Action: Inhibition of PDE4 by crisaborole leads to elevated levels of cyclic adenosine monophosphate (cAMP). Increased intracellular levels of cAMP inhibit the NF-kB pathway and suppress the release of pro-inflammatory mediators such as TNF-alfa and various interleukins that play a causative role in psoriasis and atopic dermatitis. Suppression of downstream effects in different cell types may explain the therapeutic role of crisaborole in immune-mediated skin diseases.[03]

Pharmacodynamics: Crisaborole has broad-spectrum anti-inflammatory activity by mainly targeting phosphodiesterase 4 (PDE4) enzyme that is a key regulator of inflammatory cytokine production. As this enzyme is expressed in keratinocytes and immune cells, crisaborole mediates an anti-inflammatory effect on almost all inflammatory cells. Topical application of this drug is useful as it potentiates the localization of this drug in the skin and this anti-inflammatory activity is in the low micromolar range.[04]

Absorption: Systemic concentrations of crisaborole were reached by 8 days of twice-daily topical administration. It has low systemic absorption thus poses less risk for developing systemic side effects

Metabolism: Crisaborole is substantially metabolized into inactive metabolites. The major metabolite 5-(4-cyanophenoxy)-2-hydroxyl benzylalcohol (metabolite 1), is formed via hydrolysis; this metabolite is further metabolized into downstream metabolites, among which 5-(4-cyanophenoxy)-2-hydroxyl benzoic acid (metabolite 2), formed via oxidation, is also a major metabolite.

Route of Elimination: Renal excretion of metabolites is the major route of elimination

Toxicity: Hypersensitivity reactions such as contact urticaria may occur and discontinuation of the treatment is advised. No evidence of mutagenic or clastrogenic potential as well as altered effects on fertility. Oral LD50 value for rats is >500mg/kg.

EXPERIMENTAL SECTION

Materials and Methods:

Instrumentation: A binary gradient system of 3000 series HPLC of analytical technologies ltd, was used for analysis.

Chemicals: A standard Crisaborole was procured from Pfizer pharmaceuticals pvt. Ltd. And other chemicals like methanol, water, of HPLC grade were purchased from LobaChemie, Mumbai.

Method development:

Solubility: As per literature review and solubility test the standard Crisaborole was found better soluble in Ethanol, methanol, Isopropyl alcohol.

Method: As per the trials done on methanol solvent as mobile phase, I'd got better results at 252 nm wavelength in UV-Spectroscopic method.

Method validation: A developed method was validated by some methods like Linearity, Accuracy, Precision, % Recovery, Assay, Limit of detection (LOD), Limit of Quantification (LOQ), Robustness, and Ruggedness as per ICH Guidelines.

Preparation of stock solution: 10mg of standard drug Crisaborole was dissolved in 10 ml of mobile phase solution as Methanol, it gives 1000ppm stock solution.

Preparation of test solution: Forpreparation of test solutions of 10, 20, 30, 40 & 50 ppm dilutions, I've taken 0.1, 0.2, 0.3, 0.4 &0.5 ml of solution from the stock solution of 1000 ppm and make up the volume up to 10 ml with the diluent solution respectively.

RESULTS AND DISCUSSION

Validation Parameters for method development:

Linearity:

To perform linearity I'd prepared dilutions of 10, 20, 30, 40&50 ppm concentration, and validated it on HPLC as per ICH guidelines.

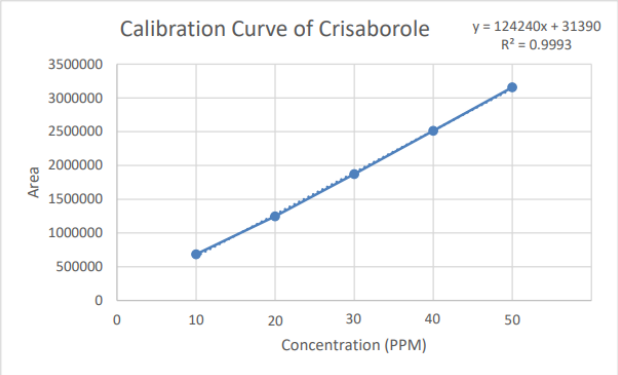

Fig2: Calibration curve of Crisaborole

Accuracy:

The accuracy of method was determined by recovery tests. So, a well-known amount of concentration of the working standard was added to fixed concentration of the pre-examined tablet solution. And the percent recovery was calculated by matching the area before and after addition of working standard. The recovery studies were done 3 times. This standard addition method was performed at 50%, 100%, 150% concentrations and the percentage recovery wascalculated. The percent recovery was within the range of 98.2 to 102.2 for Crisaborole.

Table 1- Accuracy study of Crisaborole

Sr. No.	% Composition	Sample Amount		Area of Standard	Area of Sample	Amount Recovered in ppm	% Recovery	% Mean Recovery	% SD
		Sample Amount in ppm	Amount Added in ppm						
1	50% Recovery	20	10	1873154	1886149	30.21	100.6937	100.46	0.4628
		20	10		1887329	30.23	100.7567		
		20	10		1871689	29.98	99.9218		
2	100% Recovery	20	20	2513349	2499430	39.78	99.4462	99.93	0.6060
		20	20		2513669	40.01	100.0127		
		20	20		2521980	40.13	100.3434		
3	150% Recovery	20	30	3157775	3125118	49.48	99.9159	99.96	0.8258
		20	30		3148008	49.85	99.6907		
		20	30		3166527	50.14	100.2772		

Precision:

A standard solution containing 30 ppmof Crisaborole were analyzed three times on the same day and different day, and the % RSD was calculated. The results are given in table.

Table 2- Precision study of Crisaborole

Interday Precision		Intra-day Precision	
Day 1	**Area**	**Morning**	**Area**
	4156978		4136958
	4178946		4175420
	4100365		4102158
Day 2	4186947	**Evening**	4135946
	4142695		4170231
	4136248		4102469
Mean	4150363.2	Mean	4137152
SD	28743.0183	SD	28831.7837
% RSD	0.6925%	%RSD	0.6969

Robustness:

By doing a small thoughtful change in chromatographic conditions likewise, a change in wavelength (±2 units) and flow rate (±2 units) were studied, to observe the robustness of the method. The results were found less than 2 of tailing factor of the developed RP-HPLC method for the analysis of Crisaborole. The results are given in table.

Table 3- Robustness study of Crisaborole

Level	Crisaborole	
	Retention time	**Tailing factor**

Change in	Flow rate (ml/min)	
-2(0.8ml)	5.987	1.10
0(1ml)	5.105	1.08
+2(1.2ml)	4.234	1.07
Change in	**Wavelength (nm)**	
-2(250nm)	5.107	1.10
0(252nm)	4.908	1.12
+2(254nm)	5.008	1.10

Assay:

The assay for Crisaborole tablet was performed and the % purity were calculated as follows.

%Assay =

(Sample area ÷ Standard area) × (Weight of standard ÷ Dilution of standard) × (Dilution of sample ÷ Weight of sample) × (Purity of drug ÷ 100) × (Weight of tablet ÷ Labelled claim) ×100

= (955846÷956130) × (10÷30) × (30÷10) × (100÷100) × (100÷100) × 100

=99.97%

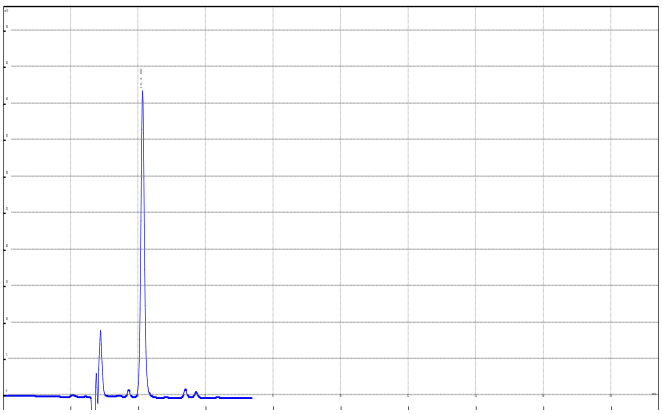

Fig 3: Chromatogram showing assay of standard injection

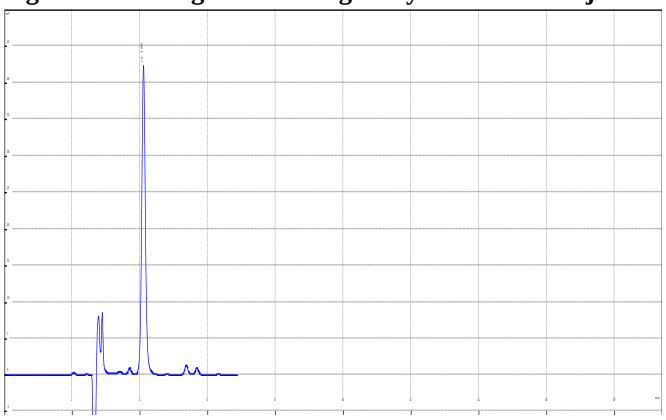

Fig 4: Chromatogram showing assay of sample injection

The % purity of Crisaborole and in pharmaceutical dosage form was found to be 99.97%

Limit of detection (LOD) and limit of quantitation (LOQ)

From linearity data, the LOD and LOQ was calculated by using formula LOD = 3.3×standard deviation ÷ Slope and LOQ = 10×standard deviation ÷ Slope. Where, standard deviation is of y intercept of linearity equations and slope is of calibration curve of the analyte. LOD and LOQ were found to be 0.3246 µg/mL and 0.9838µg/mL respectively.

CONCLUSION

The developed method was found to be simple, selective, sensitive, accurate and repeatable for analysis of Crisaborole in bulk and pharmaceutical dosage form.

REFERENCE:

1. https://Go.Drugbank.Com/Drugs/Db05219
2. https://Go.Drugbank.Com/Drugs/Db05219
3.https://Pubchem.Ncbi.Nlm.Nih.Gov/Compound/Crisaborole#Section=Metabolism-Metabolites
4. Tammisetty Mohan Rao, Ch. Balasekhara Reddy, A Validated Analytical Method For Quantification Of Crisaborole In Matrices By Uplc-Esi-Ms/Ms, Ijpsr, 2019; Vol. 10(8): 3928-3936.
5. Anna Demurtas, Silvia Pescina, Sara Nicoli, Development And Validation Of A Simple Method For The Extraction And Quantification Of Crisaborole In Skin Layers, Biomed Chromatography. 2019 Nov;33(11):E4664. Doi: 10.1002/Bmc.4664. Epub 2019 Aug 29.
6. Kurt Jarnagin, Sanjay Chanda, Dina Coronado, Vic Ciaravino, Lee T Zane, Crisaborole Topical Ointment, 2%: A Nonsteroidal, Topical, Anti-Inflammatory Phosphodiesterase 4 Inhibitor In Clinical Development For The Treatment Of Atopic Dermatitis, J Drugs Dermatol. 2016 Apr;15(4):390-6.
7. Fischer Ah, Shin Db And Margolis Dj: Racial And Ethnic Differences In Health Care Utilization For Childhood Eczema: An Analysis Of The 2001-2013 Medical Expenditure Panel Surveys. J Am Acad Dermatol 2017; 77: 1060-7
8. Hill Mk, Kheirandish Pishkenari A And Braunberger Tl: Recent Trends In Disease Severity And Quality Of Life Instruments For Patients With Atopic Dermatitis: A Systematic Review. J Am Acad Dermatol 2016; 75: 906-17. 8. 7. Luger T, Boguniewicz M And Carr W: Pimecrolimus In Atopic Dermatitis: Consensus On The Safety And The Need To Allow Use In Infants. Ped Aller Immunol 2015; 26: 306-15.

9. Margolis Dj, Abuabara K And Hoffstad Oj: Association Between Malignancy And Topical Use Of Pimecrolimus. Jama Dermatol 2015; 151: 594-9. 10. Eichenfield Lf, Tom Wl And Berger Tg: Guidelines Of Care For The Management Of Atopic Dermatitis: Section 2. Management And Treatment Of Atopic Dermatitis With Topical Therapies. J Am Acad Dermatol 2014; 71: 116-32.
11. Thomsen Sf: Atopic Dermatitis: Natural History, Diagnosis, And Treatment. Isrn Allergy 2014; 354250. 1
12. Schneider L, Tilles S And Lio P: Atopic Dermatitis: A Practice Parameter Update 2012. J Allergy Clin Immunol 2013; 131: 295-9.
13. Shaw Te, Currie Gp And Koudelka Cw: Eczema Prevalence In The United States: Data From The 2003 National Survey Of Children's Health. J Invest Dermatol 2011; 31: 67-73.
14. Silverberg Ji And Hanifin Jm: Adult Eczema Prevalence And Associations With Asthma And Other Health And Demographic Factors: A Us Population-Based Study. J Allergy Clin Immunol 2013; 132: 1132-8.
15. Boguniewicz M And Leung Dy: Atopic Dermatitis: A Disease Of The Altered Skin Barrier And Immune Dysregulation. Immunol Rev 2011; 242: 233-46.
16. Arellano Fm, Arana A And Wentworth Ce: Lymphoma Among Patients With Atopic Dermatitis And/Or Treated With Topical Immunosuppressants In The United Kingdom. J Allergy Clin Immunol 2009; 123: 1111-6.
17. Guidance For Industry. Bioanalytical Method Validation, U.S. Department Of Health And Human Services, Food And Drug Administration, Center For Drug Evaluation And Research (Cder), Center For Biologics Evaluation And Research (Cber) 2001.
18. Heyden Yv, Jimidar M, Hund E, Niemeijer N, Peeters R, Smeyers-Verbeke J, Massart Dl, And Hoogmartens J: Determination Of System Suitability Limits With A Robustness Test. J Of Chromatography A 1999; 845: 145-54.
19. Ich Harmonised Tripartite Guideline Prepared Within The Third International Conference On Harmonisation Of Technical Requirements For The Registration Of Pharmaceuticals For Human Use (Ich), Validation Of Analytical Procedures: Methodology 1996: 1-8.