WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 10, Issue 10, 19-27 Research Article ISSN 2278 – 4357

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR ANALYSIS OF CRISABOROLE

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Article Received on
30 July 2021,

Revised on 20 August 2021,
Accepted on 10 Sept. 2021

DOI: 10.20959/wjpps202110-20098

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ABSTRACT

A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Crisaborole in bulk formulation. The λmax of the Crisaborole was found to be 241nm in 10 mM Ammonium acetate buffer: ACN [60:40 % v/v]. pH 4.5. The method shows high sensitivity with linearity 5 to 25µg/ml (regression equation: y = 316606x - 427583; r^2 = 0.9992). The various parameters according to ICH guidelines and USP are followed for validating and testing of this method. The Detection limit and quantitation limit were found to be 0.1094 µg ml –1 and 0.3316 µg ml–1 respectively. The results demonstrated that the procedure is accurate, specific and reproducible (RSD <2%), and also being simple, cheap and less time consuming and appropriate for the determination of Crisaborole in bulk.

KEYWORDS: Crisaborole, HPLC method.

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. By synthetically Crisaborole is 4-[(1-hydroxy-1, 3-dihydro-2, 1-benzoxaborol-5-yl)oxy]benzonitrile and having atomic equation is C14H10BNO3. The construction of Crisaborole is appearing in fig 1.

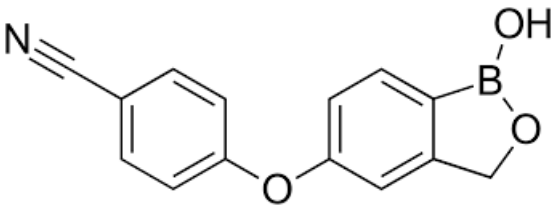

Fig. 1: Structure of crisaborole.

Crisaborole is having atomic mass of 251.05 g/mol. These nonsteroidal anti-inflammatory drugs may improve disease severity, reduce the risk of infection, and reduce signs and symptoms in people 2 years of age and older. Heal the adjacent skin and prevent the disease from getting worse with proper protection. This compound contains boron atoms that enhance skin penetration and are reliable for bimetallic contact points of phosphodiesterase-4 stimulants. This drug is currently being developed as a skin treatment for psoriasis. According to a written study, scientific strategies were calculated using HPLC and LC-MS techniques at VitalInsight. However, Liquid did not have a rational and conservative strategy. Chromatography Strategy Therefore, the purpose of this study is to develop a highly sensitive and simple HPLC strategy for evaluating crisabrol in complex structures.

MATERIALS AND METHOD

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

Drug grade unadulterated Crisaborole sample was secured from Biophore India Pharmaceutical PVT LTD. HPLC grade Methanol and water were acquired from Merck specialities private restricted, Mumbai.

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. 10 mM Ammonium Acetate buffer: ACN [60:40 % v/v].pH 4.5 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.

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).

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 10 mM Ammonium acetate buffer : ACN [60:40 % v/v].pH 4.5 utilizing C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] Table:1 and a run of the chromatograph of Crisaborole was appeared in figure 3.

Table 1: Optimized parameter.

Parameter	Condition
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	10 mM Ammonium acetate buffer : ACN [60:40 % v/v].pH 4.5
Flow Rate	1.0 ml/min
Wavelength	241 nm
Injection Volume	20 µl
Detector	UV-3000-M
Run Time	9.5 min
Retention Time	Approx. 4.2 min

Validation of RPHPLC method

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

1. 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. The calibration curves of Crisaborole sample was shown in figure 2 and their related linearity parameters given in table 2.

2. 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. A known quantity of pure drug of crisaborole was mixed to pre-analysed sample and contents again undergoes analysis by the optimised method and the % recovery was reported in table 3.

3. 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 repeatition. The results were shown in table 5.

4. 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.

5. 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 optimised 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. percentage RSD was shown in the table 7.

6. 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. Tailing factor (T) Number of theoretical plates (N) obtained was reported in table 8.

RESULT 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 (Figure 1) 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.

Table 2: Summary of results of linearity.

Conc. (µg/ml)	Peak area
5	1156321
10	2694325
15	4275906
20	6032498
25	7623154

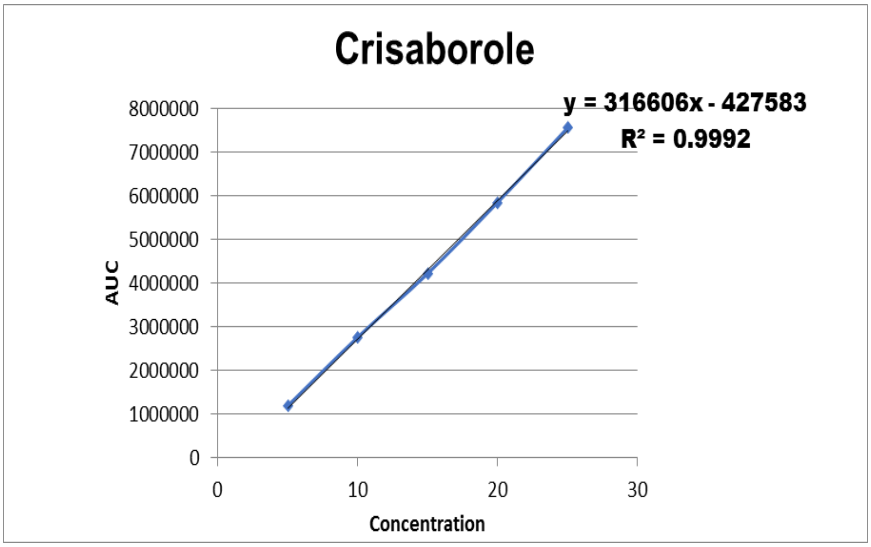

Figure 2: Linearity.

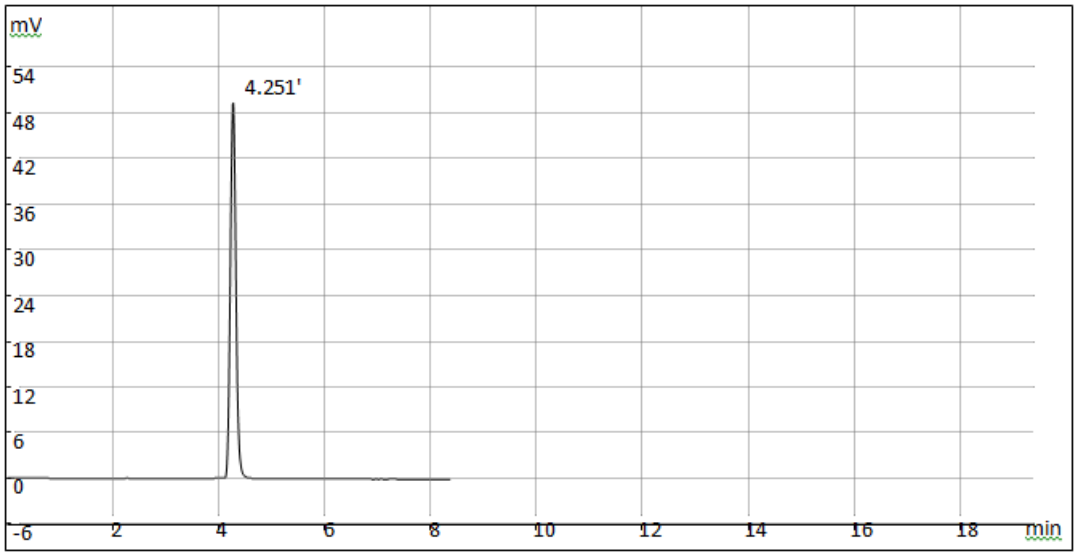

Figure 3: Typical chromatograph of crisaborole.

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 2.0% RSD, as demonstrated in Table -3.

Table 3: Summary of results of accuracy.

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
	5	10	15	4215659		14.961458	99.743051
50%	5	10	15	4203659	4226519	14.918869	99.4591294
	5	10	15	4236598		15.035771	100.23847
	10	10	20	58631789		200.22422	1001.12111
100%	10	10	20	5852136	5856613	19.984711	99.9235565
	10	10	20	5846312		19.964823	99.8241134
	15	10	25	7552169		24.960306	99.8412253
150%	15	10	25	7574812	7564179	25.035143	100.14057
	15	10	25	7594612		25.100583	100.402331

Table 4: % Recovery data.

Level of addition	% Mean recovery*	SD	% RSD
50%	99.81	0.3944	0.395161
100%	400.3	520.34	129.9897
150%	100.1	0.2808	0.280403

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 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in Table 5 and 6.

Table 5: Summary of intraday precision.

Sr. no.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	1208945			
2	5	1196317	1196841.67	11849.71	0.990082
3	5	1185263			
4	15	4236518			
5	15	4226132	4236087.67	19495.25	0.460218
6	15	4245613			
7	25	7551638			
8	25	7541623	7551958.67	10499.67	0.139032
9	25	7562615			

Table 6: Summary of interday precision.

Sr. no.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	1216354			
2	5	1202563	1214802	11541.5292	0.95007493
3	5	1225489			
4	15	4225618			
5	15	4235698	4224458	11862.6135	0.28080794
6	15	4212058			
7	25	7558963			
8	25	7589632	7563286	24472.5619	0.32357049
9	25	7541263			

LOD and LOQ

The LOD and LOQ were calculated by the equations $\text{LOD} = \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$ and $\text{LOQ} = \frac{10 \times \text{std.Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Crisaborole were 0.1094 and 0.3316 µg/ml, respectively.

Robustness

Robustness of the method reflects that the results are unaffected or reliable even if the minute changes in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 30µg/mL solution are as shown in Table No. 7.

Table 7: Robustness.

Sr. no.	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in	0.9	4236598	4221938	12736.3	0.30167
2	Flow rate	1	4213592			
3	(ml/min)	1.1	4215625			
1	Change in	239	4216523	4235580	20105.9	0.47469
2	Wavelength	241	4256592			
3	(nm)	243	4233626			

System suitability parameters

System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plate, asymmetry factor are satisfactory. The chromatographs confirm the presence of Crisaborole at 4.2 min without any interference.

Table 8: System suitability parameter.

Sr. no.	conc. (µg/ml)	Retention time	Theoretical plates	Asymmetry factor
		(min)		
1	15	4.2034	8487	1.25
2	15	4.2157	8552	1.24
3	15	4.2053	8462	1.25
4	15	4.2897	8359	1.23
5	15	4.2312	8252	1.24
6	15	4.2092	8539	1.25
Mean		4.225333333	8441.83333	1.24333333
SD		0.032776007	115.691688	0.00816497
%RSD		0.775702291	1.37045691	0.65669966

CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Crisaborole from pure and its dosage forms. The mobile phase used for method development is very simple to prepare and economical also. The sample recoveries in the formulation were showing good results. And hence, this method can be easily and conveniently adopted for routine analysis of Crisaborole in pure form.

ACKNOWLEDGEMENT

No conflict of interest.

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