Original Research Article

Method Validation of Crisaborole using Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and Quantitation of Crisaborole in Nanoemulsion Formulation for its Application in Ex Vivo Transdermal Permeation Study

Mohd Hafiz Mohd Jaafar1[1], Tommy Julianto Bustami Affendi[1], Zahid Hussain[2] and Nor Amlizan Ramli[1]*

[1]Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, Puncak Alam Campus, 42300, Selangor, Malaysia
[2]Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, University of Sharjah, UAE.

ABSTRACT

Crisaborole is a boron containing phosphodiesterase 4 (PDE4) inhibitor which in this study was formulated in nanoemulsion form to treat atopic dermatitis. The study aims to develop and validate a selective analytical method for determining crisaborole in nanoemulsion formulations, facilitating its application in *ex vivo* transdermal permeation studies for the treatment of atopic dermatitis. Crisaborole was eluted using gradient elution method in reverse phase (C18) chromatography. The mobile phases were comprised of phosphate buffer (pH3) and acetonitrile, which increasing acetonitrile volume from 30 to 95 percent within 15 min with constant flow rate of 0.65 ml/min. Column temperature was set at 40 ºC and detection wavelength was at 252 nm. The method produced linear responses (R2=0.99) in concentrations ranges from 1.56-100 µg/ml (n=7). The developed method was sensitive with limit of detection (LOD) and limit of quantitation (LOQ) values of 1.84 µg/ml and 5.58 µg/ml; respectively. Crisaborole was stable in various working conditions which were within the acceptance value provided by guidelines (±15%). In *ex vivo* studies, the concentration of crisaborole was plotted in cumulative permeated graph and this quantitative method was proven to provide a selective and sensitive measurement for detection of crisaborole.

Keywords: Crisaborole, nanoemulsion, drug carrier, chromatography, stability

Corresponding author:
Nor Amlizan Ramli
Faculty of Pharmacy
Universiti Teknologi MARA Cawangan Selangor
Puncak Alam Campus, 42300,
Selangor, Malaysia
Email: nor.amlizan@uitm.edu.my

Received: 06 Feb 2024; accepted: 15 April 2024
Available online: 30 May 2024
http://doi.org/10.24191/IJPNaCS.v7i2.02

1.0 Introduction

Crisaborole (AN2728) is a derivative of benzoxaborole which contains boron in its cyclic structure and functions as phosphodiesterase 4 (PDE4) inhibitor. Crisaborole was approved by US Food and Drug Administration (USFDA) in 2016 as a selective PDE4 inhibitor for the treatment of mild to moderate atopic dermatitis (AD) in adults and children aged 2 years old and above (1). Under pre-clinical studies, crisaborole shows no evidence of mutagenic, clastogenic or teratogenic, and no effect on rats' fertility (2,3). Mechanism of action of crisaborole is due to its tetrahedral structure that mimics the phosphate of the substrate cyclic adenosine monophosphate (cAMP) and allows crisaborole to occupy the cAMP binding site at PDE4. Inhibition of PDE4 increases cAMP accumulation subsequently triggers the activation of protein kinase A, resulting inhibition of pro-inflammatory and T cell cytokines [4]. In the clinical trial, 4.4 percent patients treated with crisaborole feels stinging or burning sensation at application site, however, the percentage was lower compared to tacrolimus (20-58%) and pimecrolimus (8-26%) (5). Upon entering systemic circulation, crisaborole lost its therapeutic effect, thus, it is beneficial to deliver crisaborole topically and retained its activity on skin layers.

In previous studies, stress test was performed on crisaborole under various condition such as acid/base hydrolysis, thermal, oxidative and photolysis to study its degradation using isocratic reverse phase method (6). Crisaborole was analyzed using several working solutions (NaCl 0.9% w/v, TFA-acetonitrile 55:45 v/v; and TFA-acetonitrile 30:70 v/v) to determine its optimal elution and accumulation of crisaborole in *in vitro* permeation study using pig skin (7). However, detection of crisaborole in extended formulations and its accumulation profile was never reported, thus this study aimed to measure crisaborole in nanoemulsion formulations and study its accumulation in *ex vivo* permeation using mice skins.

Development of nanoemulsion as drug carrier was found to be promising due to high permeation of small emulsion droplet through skin layers (8) Nanoemulsion formulation that exhibit moisturizing effects was used to hydrate the stratum corneum layer thus increasing its effectiveness (8,9). Nanoemulsion was capable to carry selective lipophilic drug with low solubility across stratum corneum into skin layer within its small oil droplets (10). Besides its high penetration ability, nanoemulsion showed high stability which results in high absorption rate, increase drug solubility and high bioavailability (11). Polymer was further used to stabilize and increased nanoemulsions' viscosity thus increased its moisturizing effect (12,13). We recently formulated nanoemulsion cream which contains tocotrienol as its main carrier and carboxymethyl cellulose (CMC) was added as stabilizer as well as increasing its moisturizing effects. With optimized formulations, we will investigate the permeability of nanoemulsion as drug carrier in comparison to control group. Olive oil was used in control group due to its hypolipidemic activity which improved in drug delivery application (14).

Therefore, in this research we established a quantitation method of crisaborole through simple methodology using reverse-phase high performance liquid chromatography (rp-HPLC) which yields simple and sensitive peaks. The method was successfully optimized to determine crisaborole concentration in nanoemulsion formulation and in *ex vivo* transdermal permeation studies. The method was validated in accordance to Bioanalytical Method Validation: Guidance for Industry (U.S Food and

Drug Administration (USFDA), Center for Drug evaluation and Research (CDER), Center for Veterinary Medicine (CVM)) based on specificity, linearity, precision, and accuracy.

2.0 Materials and Methods

2.1 Chemicals and materials

Crisaborole was purchased from Caymen Chemical Company (USA). Acetonitrile, anhydrous sodium dihydrogen phosphate and ortho-phosphoric acid (85 %) (Merck Germany), dimethyl sulfoxide (Fisher Scientific, USA), Vitamin E GOLD TRI E 50 (Sime Darby Research Sdn. Bhd., Malaysia), Cremophor RH 40 (PEG-40 hydrogenated castor oil) (BASF (Germany), Tween 80 (Zulat Pharmacy, Malaysia) and deionized water was obtained from ELGA PURELAB Option water purification system (UK).

2.2 Preparation of standard solution

A stock solution was prepared by dissolving 10 mg of crisaborole in 10 ml of dimethyl sulfoxide which yielded final concentration of 1000 µg/ml. Several concentrations of crisaborole were prepared using serial dilution ranging from 100 µg/ml – 1.56 µg/ml (n=7). Three concentrations of crisaborole were prepared as in-house quality control, which were low (1.56 µg/ml), medium (12.5 µg/ml) and high (100 µg/ml).

2.3 Preparation of mobile phase

Phosphate buffer (0.1 M, pH=3) was prepared by dissolving 12 g of anhydrous sodium dihydrogen phosphate in 250 ml of deionized water. The pH of phosphate solutions was adjusted using a diluted phosphoric acid (0.1 M) until pH 3. Finally, the final volume was brought to 1L using deionized water. Phosphate buffer and acetonitrile will be filtered and degassed prior to HPLC analysis.

2.4 Chromatographic condition

HPLC analysis was performed using Waters AQUITY HPLC system coupled with photo diode array detector. Optimum separation was achieved using Phenomenex® C18 Gemini column (150 x 4.6 mm) and mobile phase consist of acetonitrile and phosphate buffer. Gradient elution was applied by increasing concentrations of acetonitrile from 30% to 95% within 15 min with constant flowrate of 0.65 ml/min. Detection wavelength of crisaborole was set at 252 nm and the column temperature was maintained at 40°C.

2.5 Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Crisaborole standards (n=7) was injected into the HPLC system and the calibration curve were plotted by peak areas vs the concentration of crisaborole. The regression parameter (slope, intercept (c) and correlation coefficient, R^2) was calculated using linear regression analysis in Microsoft Excel (2019). The LOD and LOQ were calculated as 3.3 times the residual standard deviation of the regression line divided by the slope of the calibration curve, whereas the LOQ was calculated as 10 time the residual standard deviation divided by the slope (15).

2.6 Precision and accuracy

Accuracy was determined by recovery of crisaborole standard at three different concentration levels (low, medium, high). Samples were prepared in triplicates and analyzed for 5 times. The precision was measured by comparing triplicate sample (low, medium, high) in 6 consecutive days. All data was expressed as percent of relative

standard deviation (%RSD). The acceptance values for accuracy and precision were ±15% of nominal concentrations.

2.7 Stability

Stability of crisaborole was measured by preparing three replicates (n=3) of crisaborole samples at three different concentrations which were low (1.56 µg/ml), medium (12.5 µg/ml) and high (100 µg/ml). The stability of the samples was assessed by percentage recovery based on criterions recommended by Bioanalytical Method Validation: Guidance for Industry. The proposed criteria were (a) auto sampler condition, 24℃, 12hrs, (b) Benchtop, 24℃, 6hrs, (c) Freeze-thaw, -20 ℃, 3 cycles, (d) Frozen, -20 ℃, 30 days. The acceptance value for stability was ±15% of nominal concentrations.

2.8 Preparation of nanoemulsion

Oil in water (O/W) nanoemulsion carrier system was developed using emulsification of two immiscible phases such as oil and water. Oil phase contained vitamin E (5% w/w) as main component, PEG 40 (1.25% w/w) as main surfactant and Tween 80 (0.625% w/w) as co-surfactant. Crisaborole (2% w/w) which was lipophilic drugs was added to oil phase and stirred until dissolve at 700 rpm using magnetic stirrer. Hydrophilic phase contains water (93.125% w/w) for emulsification medium. Both phases were heated to 50ºC and stirred at 700 rpm for 30 min. Emulsification was triggered when water phase was added to oil phase and continue to heat to 50ºC and stirred at 700 rpm for 30 min. The control group were prepared by dissolving 2% w/w of crisaborole in olive oil at 50℃ at 700 rpm for 30 min using magnetic stirrer. Both nanoemulsion and control group were dispersed in 2.5% w/w CMC for 30 min at 500rpm using IKA Eurostar 20 (USA) mixer.

2.9 Ex vivo analysis on transdermal permeation of nanoemulsion using Franz diffusion cell

Male ICR strain mice (8 weeks old, 28 g) were used as animal model in permeation study using Franz diffusion cells (PermeGear, USA). Full thickness of incised dorsal skins collected were freeze for storage and thawed upon experiment. The skin was placed on the donor compartment (0.79 cm^2) and 1.3 mg of both control (2% in olive oil) and nanoemulsion (2%) cream were applied. Receptor chambers were filled with 7.5 ml phosphate buffer solution (pH7) at 37°C and stirred at 500 rpm. Samples were collected via the sampling port into the receptor chamber and sampling were conducted for 8 hours. Sample were collected (100 µl) at predetermined time (30, 60, 120, 180, 240, 300, 360, 420 and 480 min) and the samples volume taken were replaced with same amount of phosphate buffer solution. Samples were repeated in triplicate. This study was approved by The Committee on Animal Research and Ethics (UiTM CARE) with ethic approval number: UiTM CARE: 259/2019 (15/02/2019).

2.9.1 Determination of crisaborole

Optimization of HPLC method
Crisaborole nanoemulsion was prepared by spiking of 100 µg/ml of crisaborole solution into nanoemulsion formulation (1ml). The mixture was stirred for 1 min until homogenised mixture was achieved. 1ml of DMSO was added to the mixture and stirred for 1 min. Then the mixture was centrifuged at 2500 rpm for 5 min. The supernatant containing crisaborole was collected and analysed using HPLC.

Ex vivo transdermal permeation study
100 µl of samples were collected from the receptor chamber within Franz Diffusion cell at pre-determined time. Then, the

same amount (100 µl) taken was replaced with fresh PBS solutions into the receptor chamber. The samples were centrifuged at 2500 rpm for 5 min and the supernatant were introduced to HPLC for determination of crisaborole.

3.0 Results and Discussion

In Figure 1, under optimised HPLC conditions, crisaborole was observed to produce sharp and narrow peak at 9 min. As reported previously, reducing the pH of mobile phase with acid (phosphoric acid) was observe to reduce peak tailing in HPLC analysis (16). There is no other peak observed in the chromatogram indicating no interference of internal standard and reliability of the method (17).

In Figure 2, calibration curves of crisaborole standard showed a linear line over the range of 1.56 – 100 µg/ml. The data was consistent throughout the experiment which provides regression line (R^2) of 0.999 and y-interception was 2239.2. This method produces sensitive measurement of crisaborole with LOD and LOQ values of 1.84 µg/ml and 5.58 µg/ml; respectively.

Precision, the degree of repeatability of an analytical method normally expressed as the relative standard deviation (RSD) for spiked samples. There are two types of precision measurement namely intra-day and inter-day precision. Thus, three standard solutions (low, mid and high) of crisaborole ranges from 1.56 to 100 µg/ml were prepared in triplicate for an intra-day and inter-day analysis. Intra-day analysis was performed by injecting each of the three different concentrations of crisaborole three times on the same day (18). As a result, the percentage recoveries of crisaborole were 98.78% to 99.87% for all concentrations, with %RSD values were within acceptance value of ±15% (Table 1).

In inter-day analysis, samples were prepared in triplicate and analysed for 6 consecutive days. The recovery values vary from 92.49% to 105.91% for all concentrations of crisaborole and the %RSD values for inter-day analysis were within acceptance value provided in the guidelines (±15%). Both intra-day and inter-day recoveries demonstrate that the analytical method is sufficiently repeatable both intra-day and inter-day (Table 1).

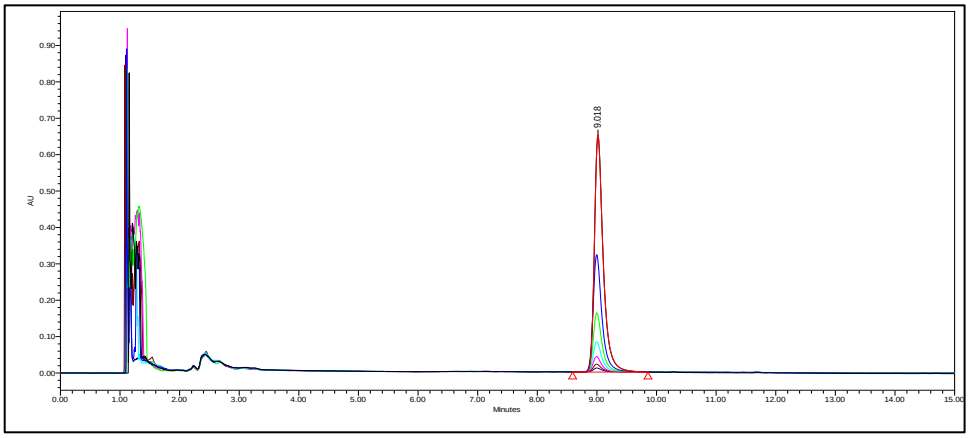

Figure 1: HPLC chromatogram of crisaborole under optimised condition with concentrations from 1.56 µg/ml to 100 µg/ml (n=7); Phenomenex C18 Gemini column (150 x 4.6 mm); gradient elution, acetonitrile from 30% to 95% within 15 min with constant flowrate of 0.65ml/min; elution time for crisaborole was at 9 min.

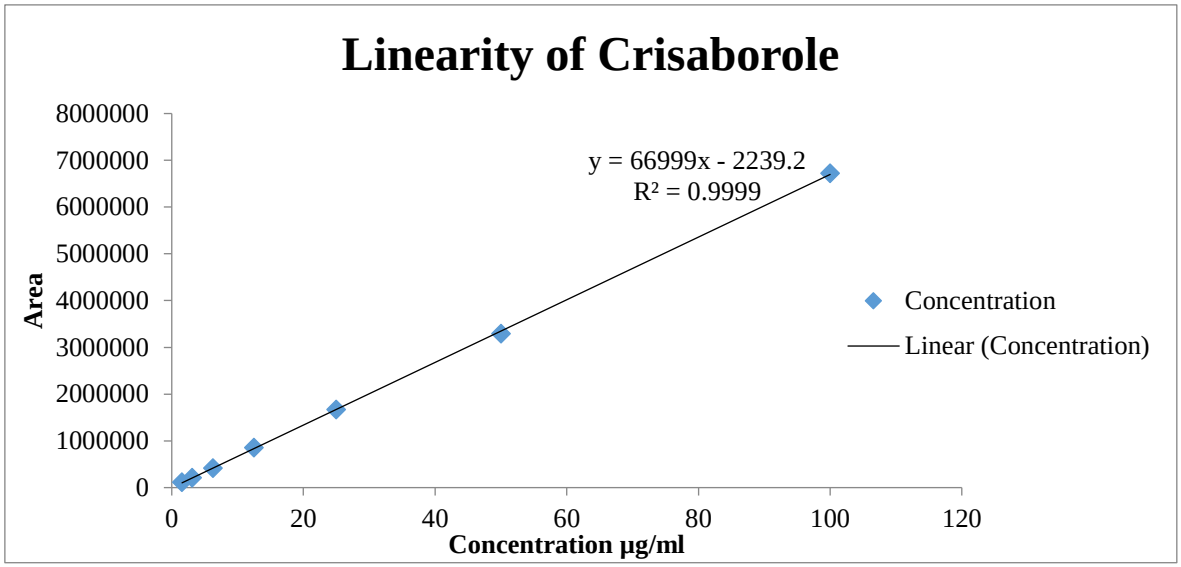

Figure 2: Calibration curve of crisaborole with concentrations of 1.56 µg/ml to 100 µg/ml n=7; linear equation was y=66999x-2239.2 with R2 of 0.9999.

Table 1: Intra-day and inter-day of crisaborole standard. Data was expressed in mean±SD and %RSD

Conc. (µg/ml)	Intra-day			Inter-day		
	Recovery	Conc.	%RSD	Recovery	Conc.	%RSD
1.56	99.87	1.56±0.07	4.78	105.91	1.65±0.08	5.03
12.5	99.64	12.45±0.20	1.63	98.21	12.28±0.43	3.50
100	98.78	98.78±0.43	0.43	92.49	92.49±4.87	5.26

Crisaborole samples were prepared and tested under various conditions to investigate its stability in normal working conditions. Benchtop and auto sampler condition reflects short storage time and normal working condition for sample preparation and HPLC analysis. Freeze-thaw cycle was performed to study the degradation of crisaborole in the presence of temperature changes from -20ºC to room temperature (24 ºC). Finally, frozen samples for 30 days indicates that, longer storage time and thermal stress will affects crisaborole degradation (6,19). In these simulated conditions shown in Table 2, shelf life and working conditions of crisaborole solutions can be determined and degradation can be avoided. Besides that, the optimal condition was established by Derringer desirability function to meet best resolution with an acceptable analytical time (20). In this study, all %RSD were within acceptance value provided by the guidelines (±15%) indicating that crisaborole is highly stable in these working conditions.

Table 2 Stability assay of crisaborole at concentrations of 1.56 to 100 µg/ml, (n=3). Data was expressed in mean±%RSD

Conditions	Mean±%RSD (n=3)		
	High	Med	Low
Autosampler, 24°C, 12hrs	98.21±0.21	12.75±1.84	1.60±10.96
Benchtop, 24°C, 6hrs	93.83±0.24	12.41±1.51	1.54±1.18
Freeze-thaw, -20°C, 3 cycles	98.41±0.22	12.52±2.22	1.57±1.89
Frozen, -20°C, 30days	90.43±1.02	12.19±1.32	1.54±4.26

Nanoemulsion was a promising drug carrier system for insoluble drug such as crisaborole due to its small size and high drug-loading capacity (21). Dispersion of hydrophobic drugs in the aqueous media improves skin permeability with good skin tolerability (22). Figure 3 showed blank nanoemulsion (vitamin E, PEG 40 and Tween 80) with no visible peak observed at 9 min indicating selectivity of this chromatographic method (23). In Figure 4, nanoemulsion spiked with 100 µg/ml of crisaborole was observed at 9 min with no interference on the peak. Therefore, this optimised chromatographic method is suitable for measuring crisaborole in nanoemulsion formulations.

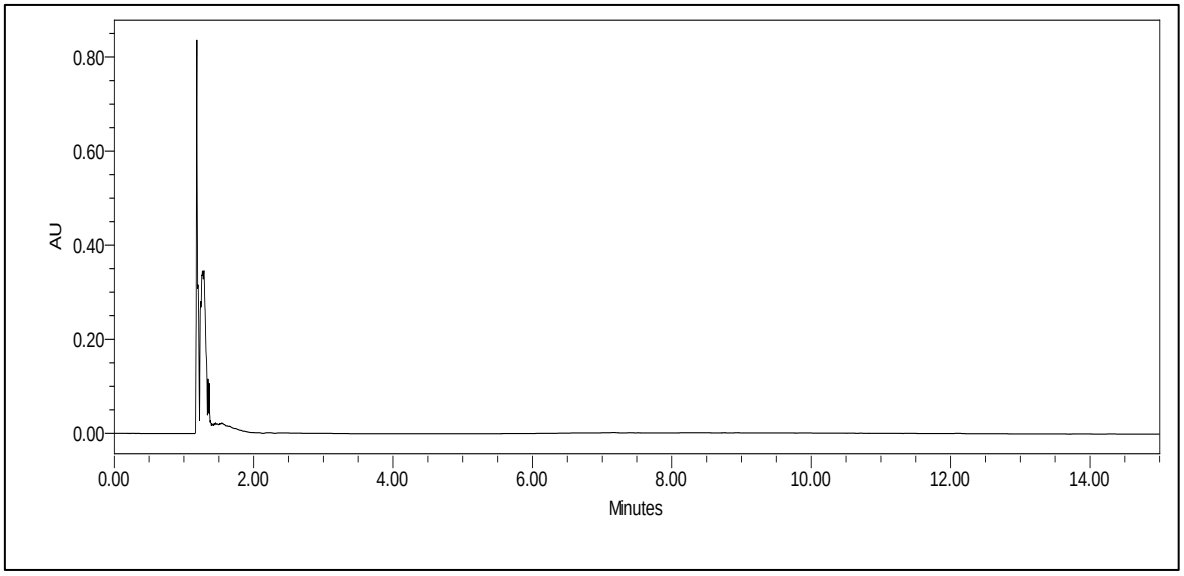

Figure 3: Chromatogram of blank nanoemulsion which contains vitamin E, PEG 40 and Tween 80.

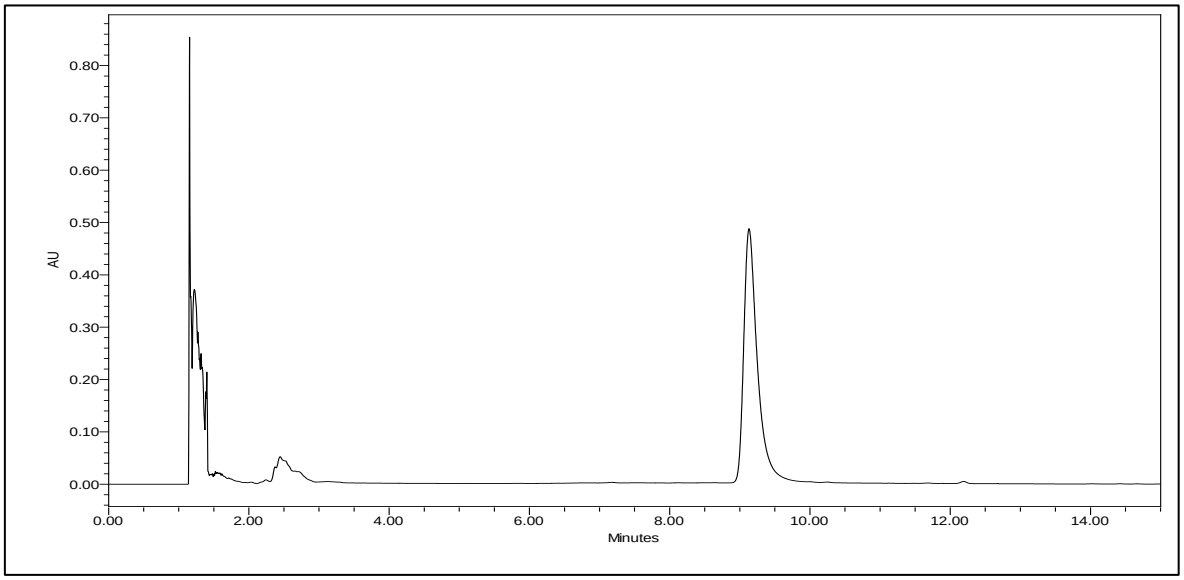

Figure 4: Chromatogram of 100 µg/ml crisaborole in nanoemulsion suspension.

Crisaborole nanoemulsion was subjected to *ex vivo* analysis using Franz diffusion cell for determination of transdermal permeation of crisaborole via nanoemulsion transport. Mice skin (ICR strain) was used as medium for permeation and receptor chamber was filled with phosphate buffer (pH7). Franz diffusion cell was widely used to measure the transdermal permeation and enhancement of nanoemulsion formulation through various tissues (19,24). DMSO was used as organic solvent to dissolve crisaborole and was found to be miscible in aqueous solution such as PBS (pH7) (25), thus suitable for quantitative analysis using HPLC.

In Figure 5, 2% crisaborole nanoemulsion and control (2% crisaborole in olive oil) were tested on mice skin to determine the permeation of crisaborole and sample were collected up to 480 min. The cumulative permeation of crisaborole profiles were plotted and both results were compared. The cumulative permeation of crisaborole in nanoemulsion group (53.50 µg/cm^2) was significantly higher as compared to control (5.58 µg/cm^2, where p=0.05). In this permeation study, determination of crisaborole was tested to achieved stable and reliable data, thus will be applied throughout the study.

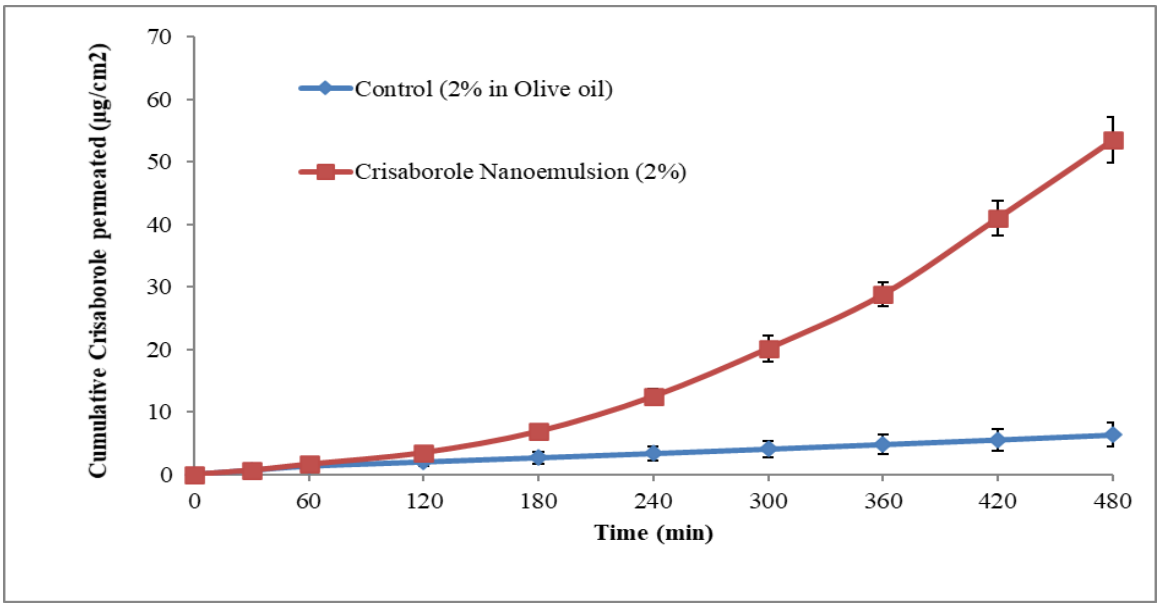

Figure 5: Transdermal profiles of crisaborole nanoemulsion (2%) as compared to control (2% crisaborole in olive oil) (n=3-5).

4.0 Conclusion

In this study, we successfully developed and validated a simple and reliable HPLC method for determination of crisaborole. The method was repeatable and selective where crisaborole peak eluted at 9 min without any interference from nanoemulsion constituents such as vitamin E, PEG 40 and Tween 80. We also determine the stability crisaborole in various working conditions without chemical degradation and within permitted values provided in guidelines. Furthermore, in *ex vivo* permeation study, the HPLC method developed was capable to quantify crisaborole in both nanoemulsion and control group and the cumulative crisaborole permeated profiles was successfully plotted. Moving forward, we will conduct *in vivo* studies on the skin to further validate the controlled release properties of the nanoemulsion.

Authorship contribution statement

MHMJ: Conducted the laboratory experiments, performed statistical analyses, and interpreted the data; **NAR**: Led the article composition, made revisions, and approved the article for submission; **TJ**: Reviewed the crisaborole nanoemulsions and their characteristics; **ZH**: Reviewed the articles and assisted with revisions.

Acknowledgment

This study was conducted in Biopharmaceutical Laboratory and Topical and Transdermal Research Laboratory, Faculty of Pharmacy, Universiti Teknologi MARA Selangor with guidance from its laboratory personnel. This study was funded by Special Research Grant, UiTM - 600-RMC/GPK 5/3 (061/2020).

Ethical Approval

This study was approved by The Committee on Animal Research and Ethics (UiTM CARE) with ethic approval number: UiTM CARE: 259/2019 (15/02/2019).

Conflict of Interest

The authors declared that they have no conflict of interest to disclose.

References

1. Zebda R, Paller AS. Phosphodiesterase 4 inhibitors. J Am Acad Dermatol. 2018;78:S43–52.

2. Wegesser T, Coppi A, Harper T, Paris M, Minocherhomji S. Nonclinical genotoxicity and carcinogenicity profile of apremilast, an oral selective inhibitor of PDE4. Regul Toxicol Pharmacol. 2021;125:104985.

3. Haverić A, Durmić-Pašić A, Alić A, Mujezinović I, Smajlović A, Ostojić J, *et al*. Biochemical and histomorphological findings in Swiss Wistar rats treated with potential boron-containing therapeutic - K2[B3O3F4OH]. J Trace Elem Med Biol. 2020;62:126642.

4. Crocetti L, Floresta G, Cilibrizzi A, Giovannoni MP. An Overview of PDE4 Inhibitors in Clinical Trials: 2010 to Early 2022. Molecules 2022;27:1–25.

5. Fahrbach K, Tarpey J, Washington EB, Hughes R, Thom H, Neary MP, *et al*. Crisaborole Ointment, 2%, for Treatment of Patients with Mild-to-Moderate Atopic Dermatitis: Systematic Literature Review and Network Meta-Analysis. Dermatol Ther (Heidelb) 2020;10:681–94.

6. Sahu PK, Panda J, Yantapalli VVJK, Piratla LLM. Stress testing of crisaborole by a novel stability indicating RP-HPLC method. SN Appl Sci. 2020;2(4):598.

7. Demurtas A, Pescina S, Nicoli S, Santi P, Padula C. Development and validation of a simple method for the extraction and quantification of crisaborole in skin layers. Biomed Chromatogr. 2019;33(11): e4664.

8. Che Marzuki NH, Wahab RA, Hamid MA. An overview of nanoemulsion: Concepts of development and cosmeceutical applications. Biotechnol Biotechnol Equip. 2019;33(1):779–97.

9. Harwansh RK, Deshmukh R, Rahman A. Nanoemulsion: Promising nanocarrier system for delivery of herbal bioactives. Int J Drug Deliv Technol. 2019;51:224-33.

10. Pandey P, Gulati N, Makhija M, Purohit D, Dureja H. Nanoemulsion: A Novel Drug Delivery Approach for Enhancement of Bioavailability. Recent Pat Nanotechnol. 2020;14(4):276–93.

11. Romes NB, Abdul Wahab R, Abdul Hamid M, Oyewusi HA, Huda N, Kobun R. Thermodynamic stability, in-vitro permeability, and in-silico molecular modeling of the optimal Elaeis guineensis leaves extract water-in-oil nanoemulsion. Sci Reports 2021;11(1): 20851.

12. Wang Y, Qu Z, Wang W, Yu D. PVA/CMC/PEDOT:PSS mixture hydrogels with high response and low impedance electronic signals for ECG

monitoring. Colloids Surf B Biointerfaces 2021;208:112088.

13. Wirjosentono B, Tamrin T, Siregar AH, Gustanto G, Nasution DA. Counseling on health and cleaning protocols, training and demonstration on preparation of hand sanitizer using betel leaf extract and carboxhymetil cellulose of rice straw as antibacterial and moisturising agents for worshipers and community around Nurul Hasanah mosque in Dusun-XI, Sei Rotan, Percut Sei Tuan, Deli Serdang Regency. AIP Conf Proc. 2021;2342(1):080014.

14. Elsewedy HS, Shehata TM, Almostafa MM, Soliman WE. Hypolipidemic Activity of Olive Oil-Based Nanostructured Lipid Carrier Containing Atorvastatin. Nanomaterials 2022;12(13):2160.

15. Gibitz-Eisath N, Eichberger M, Gruber R, Sturm S, Stuppner H. Development and validation of a rapid ultra-high performance liquid chromatography diode array detector method for Verbena officinalis L. J Pharm Biomed Anal. 2018;160:160–7.

16. Tantawi O, Baalbaki A, El Asmar R, Ghauch A. A rapid and economical method for the quantification of hydrogen peroxide (H2O2) using a modified HPLC apparatus. Sci Total Environ. 2019;654:107–17.

17. Tartaglia A, Kabir A, Ulusoy S, Sperandio E, Piccolantonio S, Ulusoy HI, *et al*. FPSE-HPLC-PDA analysis of seven paraben residues in human whole blood, plasma, and urine. J Chromatogr B Anal Technol Biomed Life Sci. 2019;1125:121707.

18. Soo Lim H, Young Hwang J, Choi EA, Lee G, Sun Yoon S, Kim MK. Development and validation of HPLC method for the determination of ferrocyanide ion in food grade salts. Food Chem. 2018;239:1167–74.

19. Fantini A, Demurtas A, Nicoli S, Padula C, Pescina S, Santi P. In Vitro Skin Retention of Crisaborole after Topical Application. Pharm. 2020;12(6):491.

20. Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. J Pharm Biomed Anal 2018;147:590–611.

21. Ghiasi Z, Esmaeli F, Aghajani M, Ghazi-Khansari M, Faramarzi MA, Amani A. Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: An in vivo study. Int J Pharm. 2019;559:341–7.

22. Barradas TN, de Holanda e Silva KG. Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. Environ Chem Lett. 2020;19(2):1153–71.

23. Tampucci S, Terreni E, Burgalassi S, Chetoni P, Monti D. Development and Validation of an HPLC-UV Method to Quantify Tavaborole During in Vitro Transungual Permeation Studies. J AOAC Int 2018;101:437–43.

24. Kurtz SL, Lawson LB. Nanoemulsions Enhance in vitro Transpapillary Diffusion of Model Fluorescent Dye Nile Red. Sci Rep. 2019;9(1):11810.

25. Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN, Arakawa T. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane. J Pharm Sci. 2020;109(1):524–31.