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DEVELOPMENT OF FORMULATION, PREPARATION PROCEDURE AND IN-HOUSE QUALITY STANDARD FOR 20% PICARIDIN CREAM AS AN INSECT REPELLENCY

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ABSTRACTS

Objectives: *This study aimed to develop a pharmaceutical formulation, and manufacturing process of a 20% picaridin cream for topical insect repellent use and to establish and validate the corresponding in-house quality standards (TCCS).*

Subjects and methods: *An oil-in-water (O/W) emulsion cream formulation containing 20% picaridin was designed. The preparation process was optimized, and the in-house quality specifications were subsequently developed and validated.*

Results: *A stable formulation and validated manufacturing procedure for a 20% picaridin cream were successfully developed. Hydroxypropyl methylcellulose (HPMC) K4M was used in aqueous phase at a concentration of 0.8%. The optimized emulsifying mixture was composed of Span 60 and Tween 80 in a 4:6 ratio. The in-house specification (TCCS) was established and included both qualitative and quantitative determination of picaridin using a validated high-performance liquid chromatography (HPLC) method, meeting criteria for accuracy, repeatability, specificity, selectivity, and linearity.*

Conclusion: *A stable 20% picaridin cream formulation (as a suitable insect repellent for military personnel) was successfully developed with required qualities outlined in the validated in-house standard.*

Keywords: Picaridin; Icaridin; cream excipients; insect repellent.

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1. INTRODUCTIONS

The prevention and control of diseases related to mosquitoes and others insects is an urgent requirement to safeguard the health and combat readiness of military personnel. In recent years, insect repellent formulations containing picaridin have been widely used worldwide due to their proven efficacy and safety profile. Studies have demonstrated that 20% picaridin provides superior protection (lasting up to 12 hours) against various harmful insects such as ticks, mosquitoes, flies, fleas,… and rodents, while ensuring safety for human use. Notably, this active ingredient is non-corrosive and does not damage common materials (e.g., fabrics, plastics, rubber, coatings, leather), making it particularly suitable for military deployment.

To date, 20% picaridin cream has been approved for its efficacy and safety by the World Health Organization (WHO), the U.S. Centers for Disease Control and Prevention (CDC), and the U.S. Environmental Protection Agency (EPA). However, there has been no published research in Vietnam on the effects of picaridin or on the formulation and manufacturing process of picaridin-containing products. Consequently, domestic production of such preparations remains limited.

For these reasons, we conducted this study to develop the formulation, manufacturing process, and in-house quality standards for a 20% picaridin cream intended for insect repellency.

2. MARTERIALS AND METHODS

2.1. Materials and equipments

- Research materials: picaridin 98.5%, Vaseline, Cetyl alcohol, Tween 80, Tween 60, Span 80, Sodium carboxymethyl cellulose (NaCMC), Hydroxypropyl methylcellulose (HPMC) K4M, HPMC E6, Glycerin, Ethanol, HPLC-grade Methanol, HPLC-grade Acetonitrile, Phosphoric acid (H_2PO_4), Formic acid, and distilled water…

- Equipments: high-speed homogenizer HG-15A (Daihan, Korea); multifunctional preparation machine (KALWEKA, India); filling machine with cooling stirrer (China); plastic tube bottom sealing machine (China); centrifuger (Hettich, Germany); high performance liquid chromatography (HPLC) system (Shimadzu, Japan); magnetic stirrer with heating function; vacuum solvent filtration apparatus; precision balance; analytical balance. All equipment met the standards for pharmaceutical formulation, production, quality control, and research, and were operated under Good Laboratory Practice (GLP) requirements.

2.2. Methods

- Formulation and manufacturing process development: A 20% picaridin oil-in-water (O/W) emulsion cream formulation was designed, consisting of two phases: the active pharmaceutical ingredient (API) - Picaridin - was dissolved in the oil phase and dispersed in the aqueous phase with emulsifying excipients. The oil phase accounted for 35% of the total formulation (including picaridin, vaseline, and cetyl alcohol). The aqueous phase made up 60% (including glycerin, NaCMC, HPMC, and distilled water). Emulsifying excipients (Span and Tween) constituted 5% of the formulation. The homogenization method was applied to blend the emulsion. Two factors responsible for their influence on the manufacturing process and on the cream's quality, stability, and consistency were evaluated: (1) gelling and bulking agents (with a focus on selecting appropriate HPMC type and concentration for the aqueous phase) and (2) hydrophilic emulsifiers.

- Evaluation criteria:

+ Visual appearance and homogeneity were assessed in accordance with the Vietnam Pharmacopoeia, Edition V.

+ Quantitative determination of picaridin was conducted using a validated high-performance liquid chromatography (HPLC) method.

+ Stability testing under stress conditions (centrifugation method at room temperature): 20 g of cream sample was placed into a test tube and centrifuged at 6,000 rpm for 5 minutes. Physical changes and phase separation were evaluated.

+ Forced degradation testing (exposure to high and low temperatures): cream samples were subjected to six cycles of temperature variation. Each cycle consisted of three stages: 24 hours at 4°C, 24 hours at 50°C, and 6 hours at room temperature. After each cycle, the samples were evaluated for quality indicators (including visual appearance, phase separation, and picaridin assay). The cream was considered acceptable if no changes were observed in any quality parameters after all six cycles.

+ Process stability assessment was based on the results of quality criteria including visual appearance, homogeneity, weight uniformity, physical stability, identification, and assay of picaridin.

- In-house standard development: The in-house specification was established based on criteria such as visual appearance, homogeneity, weight uniformity, identification and assay of API. The HPLC method for picaridin quantification was validated as part of this process.

- Data analysis: statistical analysis and comparisons were performed using Microsoft Excel, including calculation of mean values and standard deviations.

3. RESULTS

3.1. Formulation and manufacturing process

- Selection of HPMC type and concentration:

The following components were fixed: 20% picaridin, 10% vaseline, 5% cetyl alcohol, 12% glycerin, 1.2% NaCMC, 4% Tween 60, and 1% Span 60. Only the type of HPMC (either K4M or E6) and its concentration (0.4%, 0.6%, 0.8%, or 1%) were varied. A total of 8 formulations (Formulas 1–8) were prepared, each containing 100g of cream and repeated 3 times. The predetermined evaluation criteria were applied. If a sample failed to meet any one of the three critical criteria - visual appearance, homogeneity, or physical stability - the assay for API was not conducted. The results are presented in Table 1:

Table 1. Evaluation results for the selection of HPMC as a formulation excipient

Formulation (n = 3)	HPMC type	HPMC conc. (%)	Appearance	Homogeneity	Stability	Assay
1	E6	0.4	Failed	Failed	Failed	-
2	E6	0.6	Failed	Failed	Failed	-
3	E6	0.8	Passed	Passed	Failed-	-
4	E6	1.0	Passed	Passed	Passed	98.6%
5	K4M	0.4	Failed	Failed	Failed	-
6	K4M	0.6	Passed	Passed	Failed-	-
7	K4M	0.8	Passed	Passed	Passed	99.1%
8	K4M	1.0	Passed	Passed	Passed	98.9%

Table 1 shows that HPMC with higher viscosity and used at higher concentrations results in a cream of better quality. The emulsification method involving high-temperature mixing did not affect the active ingredient content in the formulation. HPMC K4M met more evaluation criteria than the other type, thus it was selected as the gelling excipient for further development.

In formula 8, the cream appeared thicker; under cold weather conditions, in combination with NaCMC, it tended to solidify, lacked smoothness, and was difficult to dispense from the tube. Therefore, the formulation used in Formula 7 (0.48 g, equivalent to 0.8% of the aqueous phase) was selected for further evaluation.

- Selection of emulsifier type and ratio:

The following components were fixed: 20% picaridin, 10% vaseline, 5% cetyl alcohol, 12% glycerin, 1.2% NaCMC, and 0.8% HPMC K4M. Only the type of Tween (Tween 60 or Tween 80) and the total emulsifier ratio (50%, 60%, or 80%) were varied. Six formulations (Formulas 9-14) were prepared and each test was repeated three times. The predefined evaluation criteria were applied. If a sample failed any of the three main criteria (visual appearance, homogeneity, or physical stability), the quantitative assay was not performed. Results are presented in Table 2.

Table 2. Results of the evaluation criteria for selecting emulsifying agents

Formulation (n = 3)	Emusifier combination	Ratio	Visual appearance	Homogeneity	Physical stability	Assay
9	Span60/Tween60	1:1	Failed	Failed	Failed	-
10	Span60/Tween60	2:3	Passed	Failed	Failed	-
11	Span60/Tween60	1:4	Passed	Passed	Passed	99.1%
12	Span60/Tween80	1:1	Passed	Passed	Failed	-
13	Span60/Tween80	2:3	Passed	Passed	Passed	98.0%
14	Span60/Tween80	1:4	Failed	Passed	Passed	-

Formulations 11 and 13 met all evaluation criteria; therefore, both were selected for further in-depth assessment.

- Forced degradation testing was conducted using 6 storage cycles (as described in section 2.2):

One representative sample from each formulation was subjected to the test to compare and evaluate the following parameters: visual appearance, phase separation, and API assay. The results are as follows:

Table 3. Result for forced degradation testing

Criteria	Formulation 11	Formulation 13
Appearance	No	No
Phase separation	Yes	No
Picaridin assay	Passed (cycle 1, 2, 3)	Passed (cycle 1-6)
Note	Phase separation at 4th cycle	No phase separation after 6 cycles

In the forced degradation test, Formulation 11 exhibited phase separation during the fourth cycle. All evaluation criteria for Formulation 13 were met across all six cycles. Thus, Formulation 13, which uses Tween 80 at 60% of the emulsifier blend (ratio 2:3), demonstrated superior emulsifying capacity, maintaining stability and cream quality under various stress conditions.

- The manufacturing process is proposed based on Formulation 13 as follows:

+ Aqueous phase preparation: soak NaCMC and HPMC K4M in distilled water to allow gel formation; incorporate glycerin into the gel to form the aqueous phase; heat the mixture in a water bath to 70°C.

+ Oil phase preparation: add vaseline, cetyl alcohol, Tween 80, and Span 60 into a beaker; heat in a water bath to 70°C; dissolve picaridin into this mixture to complete the oil phase.

+ Emulsification: gradually combine the oil phase with the aqueous phase at 65-70°C while stirring until a uniform emulsion is formed, resulting in the final picaridin cream product.

+ Stability evaluation of the manufacturing process: Three production batches were prepared, each yielding approximately 2,000g of product (equivalent to 100 tubes). The products were tested for visual appearance, homogeneity, weight uniformity, physical stability, identification and assay of API according to the established in-house specification.

The results showed that all three batches met every quality criterion, with the assay for active ingredient content yielding values close to the labeled amount (99.58%, 98.55%, and 99.51%). These findings confirm that the manufacturing process is stable and reproducible.

3.2. In-house specification development results

The in-house specification for 20% picaridin cream was developed in accordance with the general quality criteria for topical cream formulations specified in the Vietnam Pharmacopoeia, Edition V. The specification includes the following parameters: visual appearance, weight uniformity, homogeneity, identification and assay of picaridin.

Among these, the assay method was fully developed and validated using high-performance liquid chromatography (HPLC) under the following chromatographic conditions:

+ Column: C18 reversed-phase column, 150 mm length × 4.6 mm internal diameter, 5 µm particle size.

+ Detector: DAD at 210 nm.

+ Injection volume: 20 µl.

+ Flow rate: 1.0 ml/min.

+ Column temperature: laboratory temperature.

+ Mobile phase: methanol/0.1% phosphoric acid (65:35, v/v).

+ Standard and sample solution concentrations: equivalent to 0.2 mg picaridin/ml of mobile phase.

Validation results demonstrated that the method had high specificity, a linear range from 5.5 µg/ml to 487 µg/ml with R = 0.9999; accuracy ranging from 98.90% to 100.88%; and good repeatability with RSD = 0.46%.

Three production batches were prepared (each consisting of 100 tubes), and all tested parameters conformed to the proposed in-house specification. Therefore, the final proposed specification is as follows:

+ Visual appearance: the cream is uniform, white in color, has a mild fragrance, smooth texture, is free from grittiness, and does not liquefy at 37°C.

+ Weight uniformity: conforms to Vietnamese Pharmacopoeia V (Appendix 11.3).

+ Homogeneity: conforms to Vietnamese Pharmacopoeia V (Appendix 1.12).

+ Identification: using the HPLC method, the sample must show a principal peak with retention time corresponding to that of the picaridin reference standard chromatogram.

+ Assay: using the validated HPLC method as described above, the picaridin content in the final product must be between 95% and 105% of the labeled amount.

4. DISCUSSIONS

Since the late 19th century, researchers have recognized the role of insects in transmitting several dangerous diseases such as malaria, dengue fever, Zika virus,... These diseases are among the leading causes of mortality in tropical and subtropical regions. The harmful effects of vector-borne insects are well-documented; however, controlling them remains a significant challenge. In tropical countries, vector control is considered a top priority in the prevention and control of insect-borne infectious diseases.

Mosquito repellent products worldwide can be broadly classified into two categories: synthetic and natural-origin compounds. Natural mosquito repellents often work by producing smoke when burned or through passive evaporation in indoor environments. Examples include citronella, clove, mint, and geranium. Some essential oils - such as eucalyptus, peppermint, clove, and geranium - are known to have mosquito-repelling properties, but their effectiveness is short-lived due to instability when applied to the skin or burned on candles. Citronella (3,7-dimethyloct-6-en-1-al), found in lemongrass, is a common natural repellent used in various forms (candles, oils, lotions). However, these products are generally unstable and provide protection for only about two hours.

In contrast, synthetic insect repellents such as DMP, DEET, picaridin, and IR3535 are widely used for their longer-lasting effects. The first generation of synthetic repellents included dialkyl phthalates (dibutyl and dimethyl phthalate, discovered in 1929), followed by indalone (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate, in 1937) and Rutgers 612 (2-ethyl-1,3-hexanediol, in 1939). DEET was later developed and gradually replaced these earlier compounds. However, after extensive use worldwide, DEET has been associated with several adverse effects, including toxicity to plants, animals, and the environment, and the emergence of insect resistance.

Picaridin is a synthetic insect repellent developed in Europe in the 1990s and has become increasingly popular in the United States over the past decade. It is effective against a broader range of insect species and offers several advantages over DEET: it has little to no unpleasant chemical odor, produces less stickiness or greasiness, is non-damaging to clothing and plastics, and is generally safe with minimal side effects. Furthermore, it poses no significant harm to the environment or to plants and animals.

Currently, many commercial products containing picaridin are available globally in various formulations (creams, gels, ointments, solutions, suspensions), applied directly to exposed skin or used in the surrounding environment - most of which are imported. In Vietnam, however, there has been no official research published on the efficacy of picaridin, resulting in limited local production and application of such preparations. Meanwhile, the demand for effective insect repellents is very high, particularly within the military.

Therefore, the development of a 20% picaridin cream formulation, including its manufacturing process and in-house quality specification, for insect repellency - and its readiness for production and use by military personnel upon demand - is a mission of significant importance.

5. CONCLUSIONS

The study successfully developed a manufacturing process for 20% picaridin cream, with a formulation consisting of nine components: picaridin, vaseline, cetyl alcohol, glycerin, NaCMC, HPMC K4M, Span 60, Tween 80, and distilled water. An in-house quality specification was also established, covering parameters such as visual appearance, homogeneity, weight uniformity, identification and assay of picaridin. In addition, analytical methods for 20% picaridin cream were developed and validated using high-performance liquid chromatography (HPLC).

The findings of this study provide a foundation for further development, including scaling up the manufacturing process, conducting long-term stability studies to support product shelf-life determination, and evaluating the efficacy of the final product.

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