processes

MDPI

Article

The Development and Validation of a Novel HPLC-DAD Method for the Quantification of Icaridin in Insect Repellent Formulations

Fernanda Fernandes Farias [1,2], Maria Cristina Santa Bárbara [2], Valéria Adriana Pereira Martins [2], Mariana Sbaraglini Garcia Silva [2], Vanessa Cristina Martins Silva [2], Newton Andreo-Filho [3], Patricia Santos Lopes [3] and Vânia Rodrigues Leite-Silva [1,3,4,*]

1 Programa de Pós-graduação em Medicina Translacional, Departamento de Medicina, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo 04023-062, Brazil; fffarias@unifesp.br
2 Centro de Medicamentos, Cosméticos e Saneantes, Instituto Adolfo Lutz, São Paulo 01246-000, Brazil; maria.barbara@ial.sp.gov.br (M.C.S.B.); valeria.martins@ial.sp.gov.br (V.A.P.M.); mariana.sbaraglini@outlook.com (M.S.G.S.); vanessa.cmartinssilva@gmail.com (V.C.M.S.)
3 Departamento de Ciências Farmacêuticas, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo (UNIFESP), Diadema 09913-030, Brazil; newton.andreo@unifesp.br (N.A.-F.); patricia.lopes@unifesp.br (P.S.L.)
4 Frazer Institute, Faculty of Medicine, University of Queensland, Brisbane, QLD 4102, Australia
* Correspondence: vania.leite@unifesp.br

Abstract: The quality control of insect repellents contributes to the population's health since these products prevent mosquito bites and vector-borne diseases. In this study, we developed and validated a novel analytical method using high-performance liquid chromatography with a diode array detector (HPLC-DAD) for the quantification of icaridin in insect repellent lotions. The analysis was performed on a phenyl chromatographic column 150 × 4.6 mm, 3.5 µm and stabilized at 30 °C. The detection of icaridin was achieved at 4.5 min with a 20 µL injection volume of the samples. The active ingredient was extracted from the lotion samples with isopropanol and water (50:50 *v*/*v*) and then diluted to the working concentration at 0.6 mg/mL with the mobile phase. The calibration curve was linear in the concentration range of 0.1 to 1.2 mg/mL. The method was robust, specific and precise (relative standard deviations—RSD < 2%). The accuracy of the method was demonstrated by icaridin recovery. The limit of detection and quantification were 0.03 mg/mL and 0.1 mg/mL, respectively. The present report puts forward a novel analytical method for the quantification of icaridin, contributing to improving the quality control and efficacy of marketed formulations and their different presentations such as lotions, gels and sprays, demonstrating its good applicability.

Keywords: quality control; analysis; HPLC-DAD; validation; icaridin; insect repellent

check for updates

Academic Editor: Malgorzata Starek

Received: 31 December 2024
Revised: 18 February 2025
Accepted: 20 February 2025
Published: 22 February 2025

Citation: Farias, F.F.; Santa Bárbara, M.C.; Martins, V.A.P.; Silva, M.S.G.; Silva, V.C.M.; Andreo-Filho, N.; Lopes, P.S.; Leite-Silva, V.R. The Development and Validation of a Novel HPLC-DAD Method for the Quantification of Icaridin in Insect Repellent Formulations. *Processes* **2025**, *13*, 621. https://doi.org/10.3390/pr13030621

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Climate change is expanding hot and humid regions, creating ideal conditions for mosquito breeding and the spread of vector-borne viral diseases globally. Challenges in eliminating breeding sites include raising public awareness, and it is a challenge to eradicate mosquitoes near urban forests. Combining preventive measures to eliminate breeding sites with the use of repellents can help prevent mosquito bites and disease transmission [1,2].

Icaridin, also known as picaridin or KBR 3023, is a long-range-action insect repellent developed by Bayer in 1980 and available in the United States since 2005. The compound's

name according to the International Union of Pure and Applied Chemistry (IUPAC) is 1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropyl ester (Figure 1). Its molecular formula is $C_{12}H_{23}NO_3$, with a molecular weight of 229.32 g/mol and a vapor pressure of 4.43×10^{-4} mmHg at 25 °C [3]. Icaridin evaporates more slowly from the skin due to its low vapor pressure, offering a longer-lasting repellent effect compared to N,N-Diethyl-m-toluamide (DEET), the most commonly used active ingredient in repellents worldwide. While both icaridin and DEET are equally potent as insect repellents, icaridin stands out for its prolonged duration of action and lower toxicity, making it a favorable choice in many formulations [1,4–6].



Figure 1. Molecular structure of icaridin.

The effectiveness of these products is influenced by various factors, including the concentration of their active ingredients. If the concentrations are lower than stated on the label, the product's efficacy may be reduced. On the other hand, higher concentration may lead to toxicological risks. Insect repellents can exhibit varying degrees of toxicity depending on concentration and frequency of use [7,8]. This justifies the prohibition of using repellents for newborns up to 6 months of age and the cautious use of repellents during pregnancy [7]. The quality control of insect repellents as an effective regulatory action is necessary to contribute to the health of the population that uses these products.

Data from the literature show that most studies focus on detecting icaridin in environmental samples and biological matrices [9,10]. Given the typically low concentrations of active ingredients, some studies rely on high-cost methods like Liquid Chromatography–Mass Spectrometry (LC-MS) and Gas Chromatography–Mass Spectrometry (GC-MS), which are not readily available in most analytical laboratories [11]. The complexity of environmental and biological matrices, coupled with these low analyte concentrations, often necessitates advanced sample-treatment procedures [12]. Such procedures help both in sample clean-up, to eliminate potential matrix interferences, and in analyte concentration. However, these sophisticated instruments require skilled labor and extensive sample preparation, limiting their practicality for routine quality control analyses [13,14]. Although alternative methods, such as infrared and spectrophotometry, have been explored for quantifying repellent active ingredients [15], no officially published method for determining icaridin in insect repellent formulations currently exists in official compendia or the literature.

Based on the above information, this study aimed to develop and validate a high-performance liquid chromatography with diode array detector (HPLC-DAD) method for the quantification of icaridin in insect repellent lotions, in compliance with the guidelines of the International Council for Harmonisation (ICH) [16]. After validation, the method was applied to icaridin insect repellents presentations produced by different manufacturers as lotions, gel and spray as a proposal for monitoring the quality of these products in the market.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals were of analytical purity grade. The solvents acetonitrile (ACN) and isopropanol from Halogen® (São Paulo, Brazil) were obtained from Merck®-Sigma-Aldrich

(Darmstandt, Germany), both were HPLC-DAD grade solvents. Distilled water was treated to ultrapure condition using the Purilab Classic system (18.2 MΩ/cm resistivity, Elga®). The chemical reference material, the insect repellent icaridin, was imported from Shijiazhuang, China (Hebei Crovell Biotech Co. Ltd®) at 99% purity. Seven samples of insect repellents were evaluated, distributed in two lotions, two gels and three sprays from different brands, purchased from commercial establishments and online retail sites, and their qualitative compositions are presented in Table 1. For the validation phase, we used a lotion presentation (L2), due to the complexity of the formulation and the high availability of the brand in the market.

Table 1. Qualitative compositions of insect repellent formulations analyzed according to Handbook of Pharmaceutical Excipients [17].

Function	Formulations' Compositions						
	Lotion (L1)	**Lotion (L2)**	**Gel (G1)**	**Gel (G2)**	**Spray (S1)**	**Spray (S2)**	**Spray (S3)**
Active ingredient	Icaridin (7.5% m/m)	Icaridin (10.0% m/m)	Icaridin (10.0% m/m)	Icaridin (10.0% m/m)	Icaridin (5.5% m/m)	Icaridin (9.98% m/m)	Icaridin (25.0% m/m)
Emulsifier	Triethanolamine	Polysorbate 85	-	-	Polysorbate 80 Oleic acid Monostearyl propyleneglycol ether Cetearate 20 Stearate 2 Stearate 21 Palmitic acid	-	PEG 400
Solvents	Water Ethyl Alcohol	Water	Water Alcohol	Water Alcohol	-	Water Alcohol	Water Alcohol
Polymers	-	Acrylamide	-	-	Poloxamero 407	-	-
Viscosity agent	Carbomer	-	Polyacrylic Acid	Polyacrylic Acid	Docosanoic acid	-	-
Preservative	Methylparaben	-	-	-	Phenoxyethanol Caprylglycol	-	-
Emollient	Glycerol	Liquid Paraffin Ethylexyglycerin	Glycerol	Glycerol	Glycerol Caprylic triglycerol Cetyl palmitate Stearic acid	-	-
pH Regulator	Triethanolamine (alkalizing)	Sodium Hydroxide	Anhydrous Sodium Hydroxide	Anhydrous Sodium Hydroxide	-	-	-
Antioxidant	-	Tocopherol	-	-	Butylated hydroxytoluene	-	-
Fragrance	Essence Benzyl Salicylate Dextrolimonene Linalol Hydroxymethylpentyl Cycle Hexane Carboxaldehyde Citral	Benzyl alcohol	-	-	-	-	-

2.2. Instrumentation

The chromatographic analysis was performed in an HPLC-DAD apparatus (Agilent Technology, 1260 Infinity, Santa Clara, CA, USA) consisting of a binary pump (G1312B) equipped with a degasser, automatic sampler (G1329B), column compartment with an oven (G1316A) and a diode detector system (G1315D). The data were processed by Open LAB® CDS Chemstation Chromatographic Software (Version-C.01.04). We also used the following other equipment: Ultrasound Unique Ultrasonic Cleaner (Shaoguan, China) GoldSun vacuum pump model 0411 (São Paulo, Brazil), Mettler Toledo analytical balance model AL204 (Greifensee, Switzerland).

2.3. Chromatographic Conditions

This analysis was performed on a 150 × 4.6 mm, 3.5 µm Zorbax Eclipse XDB-Phenyl Agilent® phenyl chromatographic column stabilized at 30 °C (±0.8 °C). The mobile phase was acetonitrile/water (40:60 v/v) and the flow rate was kept isocratically at 1.0 mL/min at 210 nm. The injection volume was 20 µL.

2.4. Sample Preparation

One gram of the lotion sample was accurately weighed directly into a 50 mL volumetric flask and the active ingredient was extracted with 20 mL of isopropanol/water (50:50 v/v). The flask was then put into an ultrasonic bath for about 5 min for the complete extraction of icaridin, and it was completed with the same solvent. An aliquot was transferred with a volumetric pipette to another 10 mL volumetric flask to reach a concentration of 0.6 mg/mL, and completed with the mobile phase, acetonitrile/water (40:60 v/v). The aliquots were filtered through a syringe filter and subsequently injected for HPLC-DAD analysis. The same extraction pretreatment procedure was also applied to both the spray and gel samples to achieve a final concentration of 0.6 mg/mL.

2.5. Standard Solution Preparation

A total of 100 milligrams of icaridin standard were accurately weighed directly into a 50 mL volumetric flask, and 20 mL of isopropanol/water (50:50 v/v) was added. After this step, the flask was put into an ultrasonic bath for about 5 min and then the flask was completed with the same solvent, the same sample preparation to avoid any interference with the chromatogram. An aliquot was transferred with a volumetric pipette to another 10 mL volumetric flask to reach a concentration of 0.6 mg/mL, and it was completed with the mobile phase, acetonitrile/water (40:60 v/v). The aliquots were filtered through a syringe filter and subsequently injected for HPLC-DAD analysis.

2.6. Validation Parameters

After obtaining the suitable chromatographic conditions, the method was validated as per the specifications of ICH Q2 (R2) [16].

Validation of analytical procedures—scientific guidelines: Parameters such as linearity, precision (repeatability, intermediate precision), accuracy, selectivity, limit of quantification (LOQ), limit of detection (LOD) and robustness were evaluated in order to demonstrate the suitability of the method for its intended use.

3. Results and Discussion

3.1. Method Development

The detection wavelength for icaridin was determined through the spectral analysis of standard solutions prepared in different solvents. A diode array detector (DAD) scan was conducted from 190 to 440 nm to identify the wavelength corresponding to the maximum absorbance (λmax) of icaridin. Although many organic compounds, including solvents commonly used in reversed-phase HPLC, absorb in the UV range, the selection of 210 nm was based on a balance between sensitivity, specificity and chromatographic performance. To evaluate potential alternatives, we also tested 220 nm, a wavelength previously employed by Knepper [9] for detecting icaridin in aquatic environments. However, in our chromatographic conditions, the signal intensity at 220 nm was lower, likely due to differences in column chemistry, mobile-phase composition, and detector response. To support this selection, we provide a figure in the Supplementary Files illustrating the absorbance spectra of icaridin, showing its absorption intensity as a function of wavelength. Across all

the tested scenarios, icaridin consistently demonstrated strong and reproducible absorbance at 210 nm, validating its use as the optimal detection wavelength for our method.

Initially, the extraction of samples (lotion, gel and spray) was tested using different solvents at 100% concentration, including acetonitrile, ethanol and isopropanol. For most lotion samples, the resulting solution contained a significant amount of particulate matter, indicating inadequate extraction. It was observed that all the tested samples contained water in their formulations. Given that icaridin has very low solubility in water (8.6 kg/m^3), a secondary solvent, such as an alcohol, was required to form a solvent mixture capable of effectively dissolving icaridin due to its high solubility in alcohols [18].

Based on the findings of Conte et al., an isopropanol/water mixture demonstrated stability, with isopropanol being cost-effective and compatible with icaridin. This insight led to the consideration of this solvent mixture for extraction. After conducting laboratory tests and optimizing the isopropanol concentration, a 50:50 ratio of isopropanol to water was determined to be the most effective for the extraction process [18]. To confirm the complete extraction of icaridin from the samples, a known standard concentration (0.2 mg/mL) was spiked into the spray (S1) and gel (G1) formulations, yielding recoveries of 98.2% and 101.8%, respectively. The accuracy of the lotion formulation (L2) is detailed in Section 3.4.

In the initial tests, the chromatographic parameters were optimized using a C18 column with varying proportions of acetonitrile and water as the mobile phase. However, subsequent tests with a phenyl column demonstrated higher chromatographic signal intensity and enhanced performance across other evaluated parameters (Table 2). Following the injection of six replicates of a standard solution at 0.6 mg/mL, the relative standard deviations (RSDs) value was found to be below 2%, which was considered satisfactory.

Table 2. Differences in peak parameters between C18 and phenyl columns.

	C18	Phenyl
Height	310.36	430.28
Symmetry	0.94	0.97
Plates	8,161	12,707
Width	0.097	0.0642

The separation of icaridin was performed using a phenyl column under isocratic conditions, with acetonitrile and water (60:40 v/v) as the mobile phase. Therefore, this method does not use buffers in the mobile phase, which is an advantage, since buffers reduce column life. The flow rate was constant at 1.0 mL/min and the column temperature was at 30 °C. The ultraviolet (UV) wavelength was set at 210 nm, and at this wavelength, no interference from diluents, impurities or excipients present in the insect repellent formulation was observed. Before each run, the LC column was equilibrated with the mobile phase for about 30 min. A sharp and symmetrical peak was obtained for icaridin in a short run time, around 4.5 min (Figure 2).

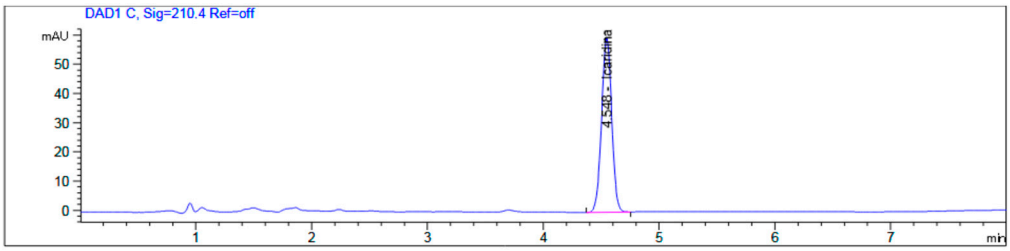


Figure 2. A chromatogram showing the icaridin peak (0.1 mg/mL) at 4.548 min, detected at 210 nm using a diode array detector.

3.2. Linearity

The linearity of an analytical procedure is the ability (within a certain range) to obtain results directly proportional to the concentration of the analyte in the sample. The analytical curve was constructed with seven concentration levels using three independent preparations. The working range was as follows: 0.1; 0.2; 0.4; 0.6; 0.8; 1.0; 1.2 mg/mL. The chromatogram of icaridin at 0.1 mg/mL at a retention time (RT) of 4.548 min demonstrates the good symmetry and sensitivity of the lowest concentration peak, as shown in Figure 2.

The absence of outliers for each concentration level analyzed (0.1 to 1.2 mg/mL) was verified using the Grubbs test. Homoscedasticity was assessed using the Cochran test, demonstrating the homogeneity of the variance of the residues. Linearity was assessed using the F test (also known as F-Snedecor) in the analysis of variance (ANOVA) of the regression. The F significance value was less than 0.05, demonstrating that the regression was significant, and the *p*-value was greater than 0.05 (0.32), that is, the data fit the simple linear model. Finally, the coefficient of determination (r^2) was 0.996.

3.3. Precision (Repeatability, Intermediate Precision)

The repeatability was investigated using the same measurement procedure, the same analyst and the same instrument under the same environmental conditions, as well as the intermediate precision, but in the last case with different analysts and days. Using the same sample preparation, six independent replicates (each corresponding to a separate sample weighing) were prepared daily with the Lotion 2 sample at a concentration of 0.6 mg/mL of icaridin, quantified against a standard of the same concentration. The means of the determinations and the RSDs were calculated. The RSD of icaridin did not exceed 2.0% for both the repeatability (1.80%) and intermediate precision (1.83%) measurements.

3.4. Accuracy

Known amounts of icaridin were added to the samples and analyzed by the proposed method. Three aliquots of the standard solutions were used to fortify sample solutions to 0.4 mL in three separate volumetric flasks. The final icaridin concentrations of these fortified solutions were 0.5, 0.6 and 0.7 mg/mL. All the solutions were prepared in triplicate and analyzed. Recovery was considered adequate once the mean recovery of icaridin calculated demonstrated values ranging from 98.2% to 101.1%.

3.5. Selectivity

The interference of the matrix on the sample was evaluated. Two analytical curves were prepared, each with identical analyte additions at all concentration levels.

One curve was prepared with analyte addition in the sample (which already contained a baseline level of the analyte), while the other curve was prepared without incorporating the sample matrix. Five concentration levels were prepared, injected in sextuplicate. The standard concentrations (Curve 1) were as follows: 0.2; 0.4; 0.6; 0.8; 1.0 mg/mL, and for the sample plus standard, they were as follows: 0.3; 0.5; 0.7; 0.9; 1.1 mg/mL, with the isolated sample at 0.1 mg/mL. The mean result of the isolated sample was subtracted from the sample plus the standard curve, and the remaining standard concentrations were presented as Curve 2 for the statistical analysis of matrix interference (Table 3).

Each point was assessed with the Grubbs test with 99% confidence, where the G maximum and minimum were less than G critical in each point. The coefficients of determination (r^2) of the curves were 1.000 and 0.9999, respectively. The results were applied to a paired *t*-test with 95% confidence, where the calculated value (0.328) was less than critical (2.045) and the *p*-value was greater than 0.5, which indicated that statistically there was no interference from the matrix.

Table 3. Statistical data on selectivity based on Grubbs' test results.

Concentration Levels (mg/mL)	Replicates	Results (mg/mL)		Descriptive Analysis			Grubbs Test with 99% Confidence	
		Curve 1	Curve 2		Curve 1	Curve 2		Curve 1	Curve 2
0.2	1	0.2016	0.2037	Mean	0.2025	0.2037	G critical	1.973	
	2	0.2025	0.2035	SD	0.0005	0.0001	G maximum calculated	0.920	1.297
	3	0.2029	0.2038	RSD%	0.2279	0.0696	G minimum calculated	1.901	1.297
	4	0.2026	0.2036						
	5	0.2024	0.2036						
	6	0.2027	0.2039						
0.4	1	0.4039	0.4040	Mean	0.4038	0.4033	G critical	1.973	
	2	0.4038	0.4040	SD	0.0003	0.0006	G maximum calculated	1.039	1.091
	3	0.4034	0.4033	RSD%	0.0765	0.1415	G minimum calculated	1.436	1.339
	4	0.4041	0.4028						
	5	0.4041	0.4034						
	6	0.4035	0.4026						
0.6	1	0.6048	0.6055	Mean	0.6051	0.6026	G critical	1.973	
	2	0.6047	0.6032	SD	0.0004	0.0019	G maximum calculated	1.117	1.552
	3	0.6053	0.6030	RSD%	0.0725	0.3157	G minimum calculated	0.945	1.215
	4	0.6047	0.6028						
	5	0.6056	0.6003						
	6	0.6056	0.6007						
0.8	1	0.8042	0.8002	Mean	0.8034	0.7996	G critical	1.973	
	2	0.8054	0.7995	SD	0.0018	0.0004	G maximum calculated	1.104	1.703
	3	0.8044	0.7994	RSD%	0.2287	0.0461	G minimum calculated	1.715	1.293
	4	0.8036	0.7991						
	5	0.8024	0.7997						
	6	0.8002	0.7997						
1.0	1	1.0071	1.0087	Mean	1.0049	1.0094	G critical	1.973	
	2	1.0047	1.0094	SD	0.0018	0.0009	G maximum calculated	1.258	1.552
	3	1.0020	1.0107	RSD%	0.1791	0.0846	G minimum calculated	1.572	1.331
	4	1.0037	1.0083						
	5	1.0059	1.0098						
	6	1.0057	1.0095						

Also, tests were made with common excipients present in the tested formulations to evaluate if there were any interferences. The excipients that exhibited relevant peaks had retention times distinct from that of icaridin, including Carbomer (0.9 min), Methylparaben (3.6 min) and Polysorbate 85 (5.4 min). Additionally, Triethanolamine, Liquid Paraffin and Glycerol were evaluated and confirmed to have no peaks overlapping with icaridin's retention time (Figure 3).

Figure 4 demonstrates that the purity factor is within the calculated threshold limit. The spectral homogeneity of a chromatographic peak, indicatives of its chromatographic purity, was demonstrated through evaluation via Open LAB® CDS Chemstation Chromatographic Software (Version-C.01.04).

The sample was further analyzed by LC-MS, using the same column and solvent proportion as the current method, with 5 mM ammonium formate ($HCOONH_4$)/0.1% formic acid (HCOOH). The temperature was 40 °C, with a flow of 0.6 mL/min and an injection volume of 2 µL. The spectrometry method conditions are presented in Table 4.

Only icaridin's peak was observed in the chromatogram sample analysis, appearing at 8.4 min (Figure 5). When protonated in the electrospray source, in positive mode, icaridin acquires a proton and starts to present an m/z ratio of 230.1100.

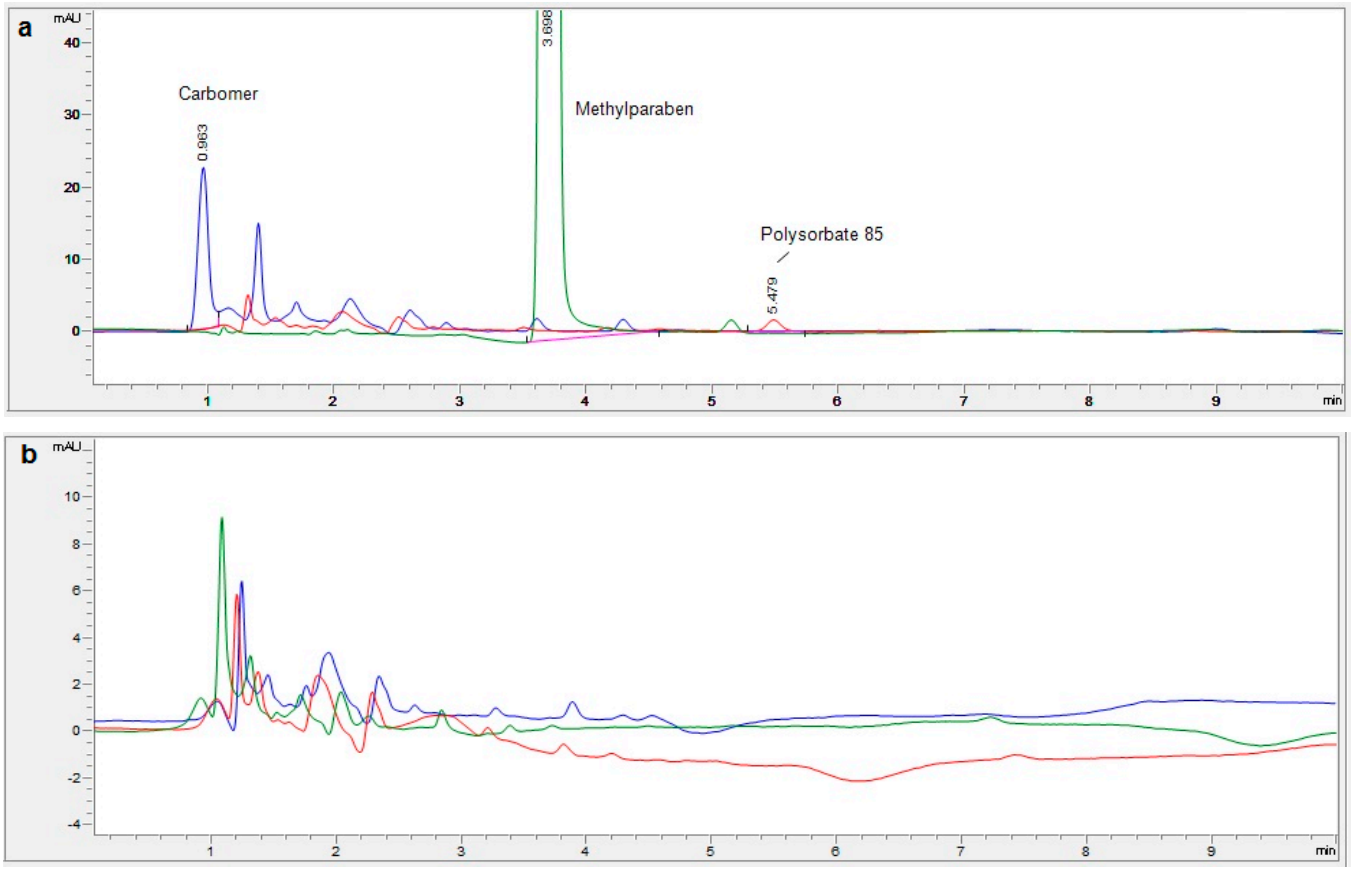


Figure 3. (**a**) Carbomer, Methylparaben and Polysorbate 85 identification and chromatogram analysis. (**b**) Triethanolamine (blue), Liquid Paraffin (red), and Glycerol (green) chromatogram analysis.

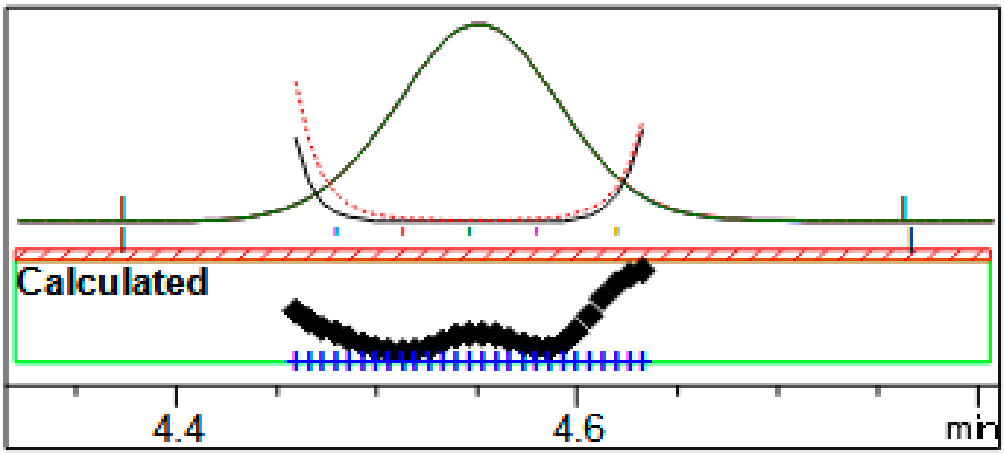


Figure 4. Peak purity curve of icaridin at 4.5 min.

Table 4. Mass spectrometry method conditions analyzed by LC-MS.

Mass Spectometry Conditions	
Equipment	QQQ Mass Spectrometer
Ionization Mode	ESI
Polarity	+
Fragmentor (V)	75
Gas Temperature (°C)	350
Gas Flow (L/min)	10
Nebulizer (psi)	35
Start and End Mass (m/z)	50–700 scan mode
Capilar Voltage (V)	4000
Sheath Gas Temperature (°C)	400
Sheath Gas Flow (L/min)	12
Nozzle Voltage (V)	1500

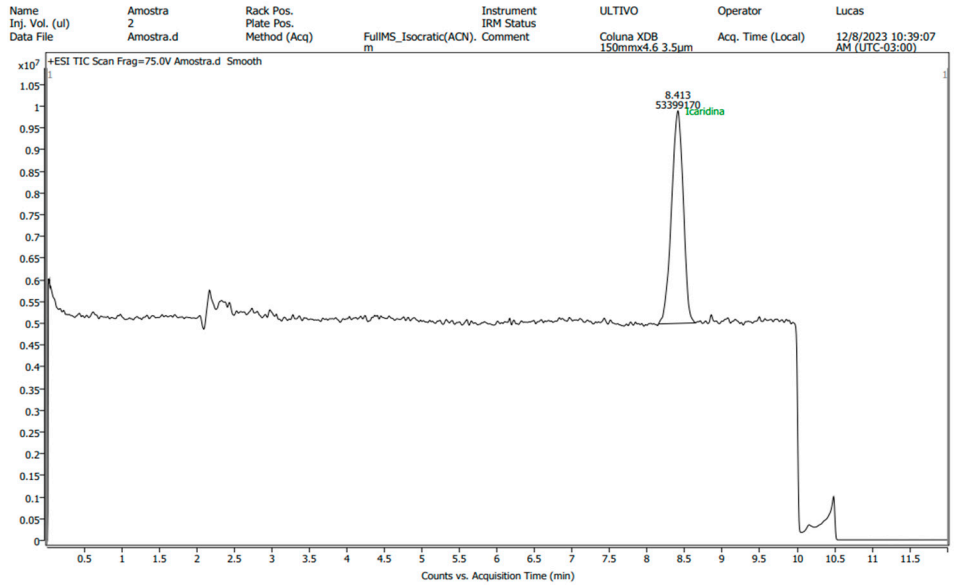


Figure 5. Icaridin sample chromatogram in LC-MS.

Therefore, Figure 6 corroborates the molecular mass of the protonated asset. The m/z of 212.11 m/z is likely a hydrolysis of protonated icaridin. The m/z of 174.11 m/z refers to the $C_8H_{16}NO_3$ molecule, a known fragmentation of icaridin in the literature [19]. The 252 m/z signal may correspond to icaridin with a sodium adduct (229 + 23). The formation of adducts is common in LC-MS, and the analyzed lotion formulation (L2) contains sodium hydroxide as an excipient.

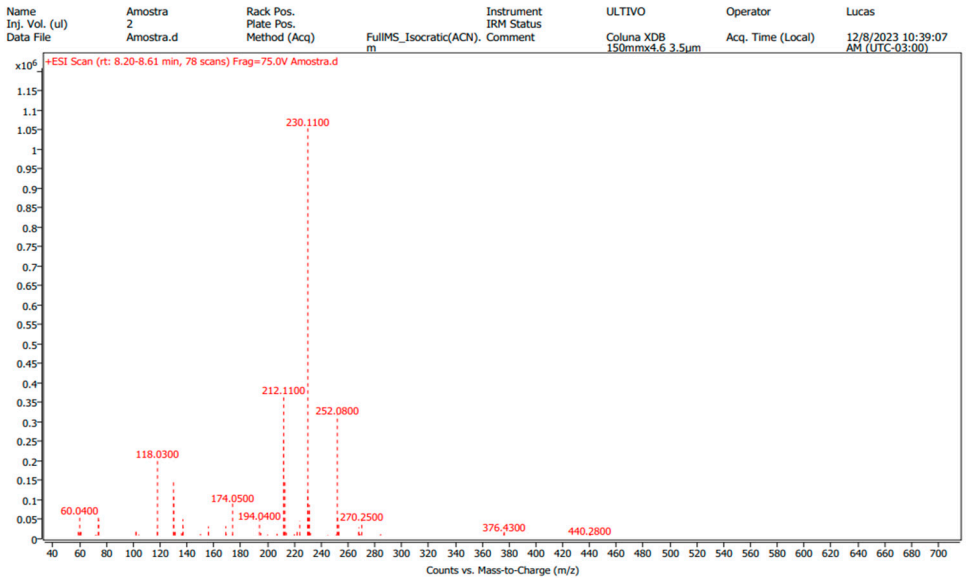


Figure 6. Mass spectrum in scan mode, ESI+ ionization of icaridin in sample, m/z ratio 230.1100.

3.6. Limit of Quantification and Limit of Detection

The LOD represents the lowest quantity of analyte that can be detected in a sample preparation, though it may not be accurately quantified under current experimental conditions. The LOQ is defined as the lowest concentration on the standard curve that can be quantified with acceptable accuracy, precision and variability [16]. For this study, these limits were determined according to one of the approaches established by the ICH [16], using the Based on Visual Evaluation method, in which the limit is determined by analyzing samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected and quantified. Successive dilutions were performed until

the lowest concentration was identified that could be quantified with confidence. After performing successive dilutions of the standard icaridin solution, the lowest concentration at which independent analysis at the same concentration level exhibited an RSD below 2% was established as the LOQ. This value was determined to be 0.1 mg/mL, as shown in Figure 2, which also serves as the first point of the linearity curve. The LOD was calculated by dividing the LOQ by 3.3, resulting in an LOD of 0.033 mg/mL.

3.7. Robustness

Robustness was evaluated by varying the column oven temperature by ±1 °C (analyses at 29 °C and 31 °C), flow rate by ±0.1 mL/min (0.9 and 1.1 mL/min) and detector wavelength by ±1 nm (209 and 211 nm). A single-factor ANOVA was applied to each group to evaluate the results. The statistical data demonstrated that there were no significant differences between the results of icaridin content in the chromatographic conditions of the validated method when compared with the content after the intentional variations. The calculated F was less than the critical F and the *p*-value was greater than 0.05, demonstrating that it is a robust method for the analyzed conditions. The parameters mentioned above were tested and validated according to acceptance criteria (ICH) specified for each one, and the results are presented in Table 5.

Table 5. Results of validation parameter tests according to ICH Q2 (R2).

Parameter	Acceptance Criterion	Result
Linearity	$r^2 > 0.99$	$r^2 = 0.996$
Repeatability	RSD ≤ 2.0%	RSD = 1.80%
Intermediate precision	RSD ≤ 2.0%	RSD = 1.83%
Accuracy	98.0 to 102.0%	Concentrations of 0.5; 0.6; 0.7 mg/mL Ranging from 98.2 to 101.1%
Selectivity	Absence of matrix interference and chromatographic purity	Matrix did not interfere with sample reading Homogeneous slopes Pure peak
Limit of quantification	Lowest concentration that shows repeatability compliant	0.1 mg/mL
Limit of detection	Lowest amount of analyte that can be detected in a sample	0.03 mg/mL
Robustness	Variation in temperature, flow and wavelength	One-way ANOVA No significant differences Wavelength: *p*-value: 0.99 Flow: *p*-value: 0.37 Temperature: *p*-value: 0.48

RSD: relative standard deviation; r^2: coefficient of determination.

3.8. Applicability of Validated Method

According to ANVISA specifications [20], the maximum allowed variation is 10% or less of the nominal value of icaridin declared on the product label. All samples were in accordance with specifications; therefore, they were considered satisfactory, as shown in Table 6.

Table 6. Description of icaridin contents and variation according to ANVISA specifications.

Formulation Type	Content Described	Content Observed	Variation Allowed
Lotion (L1)	7.50%	8.18%	6.75–8.25%
Lotion (L2)	10.00%	10.93%	9.00–11.00%
Gel (G1)	10.00%	9.87%	9.00–11.00%
Gel (G2)	10.00%	9.92%	9.00–11.00%
Spray (S1)	5.50%	5.84%	4.95–6.05%
Spray (S2)	9.98%	10.85%	8.98–10.98%
Spray (S3)	25.00%	25.96%	22.50–27.50%

Detailed articles on biocide analysis in environmental samples or blood have been published [9,10,21,22]. However, there is a notable absence of studies specifically focused on the analysis of icaridin in insect repellent formulations.

The method developed in this study does not require complex sample treatment, and uses HPLC-DAD, a method with equipment commonly available at quality control laboratories, making it a highly accessible method. To the best of the authors' knowledge, there are no reports detailing analytical methods to assay icaridin in spray, lotion and gel formulations.

Proposing a rapid and effective method, with a good resolution, offers a viable and efficient means for the quality control of repellent products on a large scale, which is still lacking in oversight. The proposed method proved to be fast, effective, accurate and viable for quality control of these products.

4. Conclusions

An HPLC-DAD method for identifying icaridin was developed and validated. The results showed that the method is very specific, accurate, with percentage recoveries between 98 and 101%, and reproducible, with the RSD < 2%. The method involves the use of a simple mobile phase and minimal sample preparation, encouraging its application in quality control for the analysis of insect repellent product formulations. Moreover, the retention time was also short (<5 min). The systematic evaluation of the method through experimental trials and its applicability to other presentations provided objective evidence that the specific requirements for its intended were met. Thus, the application of this method for the quality control for repellents containing icaridin can help ensure compliance with legislation and protect consumer rights.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr13030621/s1, Figure S1: Maximum absorption of icaridin at 210 nm; Figure S2: Chromatograms of icaridin detected at 4.5 min in analysis of real samples.

Author Contributions: F.F.F.: Conceptualization, methodology, validation, data curation, writing—original draft. M.C.S.B.: Formal analysis, writing—review and editing. V.A.P.M.: Resources, writing—review and editing. M.S.G.S.: Validation, data curation, writing—review and editing. V.C.M.S.: Visualization, writing—review and editing. N.A.-F.: Resources, writing—review and editing. P.S.L.: Resources, writing—review and editing. V.R.L.-S.: Conceptualization, supervision, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundo Especial de Saúde para Imunização em Massa e Controle de Doenças (FESIMA), of the Secretaria de Estado Da Saúde (SES), grant number (CAF n: 071/2024), The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the Productivity Scholarship in Technological Development and Extension Innovation—DT (CNPq—Process 302153/2023-3) and FAPESP (n. 2024/12480-1).

Data Availability Statement: Data are available on request from the authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tavares, M.; da Silva, M.R.M.; de Oliveira de Siqueira, L.B.; Rodrigues, R.A.S.; Bodjolle-d'Almeida, L.; dos Santos, E.P.; Ricci-Júnior, E. Trends in insect repellent formulations: A review. *Int. J. Pharm.* **2018**, *539*, 190–209. [CrossRef] [PubMed]
2. Vilar, W.T.S.; Sousa, E.S.; Pinto, L.; Ugulino De Araújo, M.C.; Coelho Pontes, M.J. Development and validation of a HPLC method to quantify DEET and IR3535 in insect repellents. *Anal. Methods.* **2018**, *10*, 1911–1917. [CrossRef]
3. National Center for Biotechnology Information. Icaridin. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Icaridin (accessed on 5 August 2024).

4. Departamento Científico de Dermatologia. Repelentes e Outras Medidas Protetoras Contra Insetos na Infância. In *Guia Prático de Atualização SBP*; SBP: Curitiba, PR, Brazil, 2020; pp. 1–11.
5. de Cataldi, D.G. Icaridina Como Ativo Repelente de Insetos: Uma Revisão Sobre sua Toxicologia e Eficácia. Bachelor's Thesis, FATEC Luigi Papaiz, Diadema, SP, Brazil, 2021.
6. dos Santos, J.; Lourenço, R.L.; Rosa, P.; Adams, A.I.H. Development and Validation of a Simple HPLC-UV Method to Assay DEET Repellents and its Application to Different Commercial Forms. *Curr. Pharm. Anal.* **2020**, *17*, 1051–1059. [CrossRef]
7. Charlton, N.P.; Murphy, L.T.; Parker Cote, J.L.; Vakkalanka, J.P. The toxicity of picaridin containing insect repellent reported to the National Poison Data System. *Clin. Toxicol.* **2016**, *54*, 655–658. [CrossRef]
8. Antwi, F.B.; Shama, L.M.; Peterson, R.K.D. Risk assessments for the insect repellents DEET and picaridin. *Regul. Toxicol. Pharmacol.* **2008**, *51*, 31–36. [CrossRef] [PubMed]
9. Knepper, T.P. Analysis and mass spectrometric characterization of the insect repellent Bayrepel and its main metabolite Bayrepel-acid. *J. Chromatogr. A.* **2004**, *1046*, 159–166.
10. Chen, Z.F.; Ying, G.G.; Lai, H.J.; Chen, F.; Su, H.C.; Liu, Y.S.; Peng, F.Q.; Zhao, J.L. Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2012**, *404*, 3175–3188. [CrossRef] [PubMed]
11. Sahu, P.K.; Ramisetti, N.R.; Cecchi, T.; Swain, S.; Patro, C.S.; Panda, J. An overview of experimental designs in HPLC method development and validation. *J. Pharm. Biomed. Anal.* **2018**, *147*, 590–611. [CrossRef] [PubMed]
12. Jiménez-Díaz, I.; Zafra-Gómez, A.; Ballesteros, O.; Navalón, A. Analytical methods for the determination of personal care products in human samples: An overview. *Talanta* **2014**, *129*, 448–458. [CrossRef] [PubMed]
13. Vilar, W.; Barbosa, M.; Pinto, L.; de Araújo, M.; Pontes, M. Determination of N, N-diethyl-3-methylbenzamide and ethyl-butyl-acetylaminopropionate in insect repellent using near infrared spectroscopy and multivariate calibration. *Microchem. J.* **2020**, *152*, 104285. [CrossRef]
14. Araya-Sibaja, A.M.; Fandaruff, C. N,N-diethyl-meta-toluamide (DEET) in repellent solutions: Development and validation of an analytical method. *Rev. Bras. Farm.* **2013**, *94*, 273–278.
15. Silva, D.I.O.; Vilar, W.T.S.; Pontes, M.J.C. Chemometric-assisted UV spectrophotometric method for determination of N, N-diethyl-3-methylbenzamide in insect repellents. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **2020**, *241*, 118660. [CrossRef]
16. Validation of Analytical Procedures. ICH Harmonised Tripartite Guideline. ICH Q2(R2). Available online: https://database.ich.org/sites/default/files/ICH_Q2-R2_Document_Step2_Guideline_2022_0324.pdf (accessed on 18 November 2024).
17. Sheskey, P.J.; Hancock, B.C.; Moss, G.P.; Goldfarb, D.J. *Handbook of Pharmaceutical Excipients*, 9th ed.; Pharmaceutical Press: Chicago, IL, USA, 2020; pp. 1–1400.
18. Conte, E.; Gani, R.; Cheng, Y.S.; Ng, K.M. Design of formulated products: Experimental component. *AIChE J.* **2012**, *58*, 173–189. [CrossRef]
19. Mass Bank-Icaridin Mass Spectrum. Available online: https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-BAFG-CSL23111019052 (accessed on 5 October 2024).
20. Nota Técnica No. 01/2018-GHCOS/DIARE/ANVISA. Available online: https://www.gov.br/anvisa/pt-br/setorregulado/regularizacao/cosmeticos/notas-tecnicas/esclarecimentos-para-o-registro-de-repelentes-de-insetos/view (accessed on 22 October 2024).
21. Wluka, A.K.; Rüdel, H.; Pohl, K.; Schwarzbauer, J. Analytical method development for the determination of eight biocides in various environmental compartments and application for monitoring purposes. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 21894–21907. [CrossRef] [PubMed]
22. Schettgen, T.; Bertram, J.; Weber, T.; Kraus, T.; Kolossa-Gehring, M. Quantification of a mercapturate metabolite of the biocides methylisothiazolinone and chloromethylisothiazolinone ("M-12") in human urine using online-SPE-LC/MS/MS. *Anal. Methods* **2021**, *13*, 1847–1856. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.