Development and application of analytical method for quantification of zinc pyrithione in shampoos by UV/VIS spectroscopy

Desenvolvimento e aplicação de método analítico para quantificação de piritionato de zinco em xampus por espectroscopia UV/Vis

DOI:10.34119/bjhrv5n2-176

Recebimento dos originais: 14/01/2022
Aceitação para publicação: 28/02/2022

Gláucia Laís Nunes Lopes
Mestre em Ciências Farmacêuticas
Instituição: Universidade Federal do Piauí
Endereço: Campus Universitário Ministro Petrônio Portella, Ininga - CEP: 64049-550
Teresina - PI
E-mail: glaucialaisnl@yahoo.com.br

Laynne Hellen de Carvalho Leal
Especialista em Alta Complexidade Hospitalar pelo Programa de Residência Multiprofissional
Instituição: Universidade Federal do Piauí
Endereço: Campus Universitário Ministro Petrônio Portella - Ininga - CEP: 64049-550
Teresina - PI
E-mail: lhcleal@hotmail.com

Naiane Carvalho Nogueira
Mestre em Ciências Farmacêuticas
Universidade Federal do Piauí
Endereço: Campus Universitário Ministro Petrônio Portella - Ininga - CEP: 64049-550
Teresina - PI
E-mail: nai.cn@hotmail.com

Iwyson Henrique Fernandes da Costa
Especialista em Transplante pelo programa de Residência Integrada Multiprofissional, Hospital das Clínicas da UFMG - Universidade Federal de Minas Gerais - Campus saúde
Instituição: Universidade Federal do Ceará
Endereço: Av. Prof. Alfredo Balena, 110, Santa Efigênia - CEP: 30130-100
Belo Horizonte - MG
E-mail: iwyson@gmail.com

Shayara Lopes Ciríaco
Mestre em Ciências Farmacêuticas
Instituição: Universidade Federal do Piauí
Endereço: Campus Universitário Ministro Petrônio Portella, Ininga - CEP: 64049-550
Teresina - PI
E-mail: shayaraciriaco@hotmail.com

André Luís Menezes Carvalho
Doutor em Ciências Farmacêuticas pela Universidade Federal de Pernambuco
Instituição: Universidade Federal do Piauí
Endereço: Campus Universitário Ministro Petrônio Portella, Ininga - CEP: 64049-550
Teresina - PI
E-mail: aluismenezes@ufpi.edu.br

ABSTRACT
Objective: develop and evaluate an analytical methodology using uv/vis spectroscopy for quantification of industrialized and compounded shampoos, and perform a physicochemical characterization in the products, including the content of zinc pyrithione. Methods: the validation of the analytical method was performed following the parameters of specificity and selectivity, linearity, precision, accuracy, and robustness established by anvisa in rdc nº 166/2017, validation of analytical procedures: text and methodology (complementary guideline on methodology dated 6 november 1996 incorporated in november 2005) and analytical procedures and methods validation for drugs and biologics (fda). The following assays of physical-chemical characterization were performed for both zinc pyrithione shampoo and placebo formulation: organoleptic characterization, ph, conductivity, content, foam index and viscosity. Results: the method was precise, repeatability obtained cv% between 3.71 and 3.91%, complying with the resolution. The study of intermediate precision also was in accordance with the resolution, which value obtained for cv% was between 3.17 and 3.79%. Results did not show statistically significant differences ($p<0.005$). Accuracy had a slight variation, regarding to 100% of active principle. The method was considered robust. Sample a content presented a concentration of zinc pyrithione inferior to the recommended in the literature. Conclusion: the presented methodology showed to be simple and cheap through uv/vis-spectrophotometry. It is important to characterize the final product in order to provide the desired effect.

Keywords: chemical analysis, dandruff, hair treatment, quality control, shampoo, UV/VIS spectroscopy.

RESUMO
Objetivo: desenvolver e avaliar uma metodologia analítica utilizando espectroscopia uv/vis para quantificação de xampus industrializados e compostos, e realizar uma caracterização físico-química nos produtos, incluindo o conteúdo de zinco piritiona. Métodos: a validação do método analítico foi realizada seguindo os parâmetros de especificidade e seletividade, linearidade, precisão, exatidão e robustez estabelecidos pela anvisa no rdc nº 166/2017, validação de procedimentos analíticos: texto e metodologia (diretriz complementar sobre metodologia de 6 de novembro de 1996 incorporada em novembro de 2005) e procedimentos e métodos analíticos de validação para drogas e produtos biológicos (fda). Os seguintes ensaios de caracterização físico-química foram realizados tanto para o xampu de zinco piritiona quanto para a formulação de placebo: caracterização organoléptica, ph, condutividade, conteúdo, índice de espuma e viscosidade. Resultados: o método foi preciso, repetibilidade obtida cv% entre 3,71 e 3,91%, cumprindo com a resolução. O estudo de precisão intermediária também estava de acordo com a resolução, cujo valor obtido para cv% estava entre 3,17 e 3,79%. Os resultados não mostraram diferenças estatisticamente significativas ($p<0,005$). A precisão teve uma pequena variação, em relação a 100% do princípio ativo. O método foi considerado robusto. Uma amostra de um conteúdo apresentou uma concentração de zinco piritiona inferior à recomendada na literatura. Conclusão: a metodologia apresentada mostrou ser simples e barata através da uv/vis-espectrofotometria. É importante caracterizar o produto final a fim de proporcionar o efeito desejado.

Palavras-chave: análise química, caspa, tratamento capilar, controle de qualidade, xampu, espectroscopia UV/VIS.

1 INTRODUCTION

Dandruff is a common complaint that affects as much as half of the population at some time post puberty. The condition is generally characterized by the presence of flakes on the scalp and in the hair, and is often accompanied by itch. The use of an anti-dandruff shampoo (AD) is one of the most applied home remedies for the treatment of dandruff. The active ingredients of AD shampoos are anti-fungals, one of them is zinc pyrithione (ZPT) [1]. The use of ZPT in antidandruff preparations has required that analytical techniques become available for quality control purposes during manufacture [2]. Method validation is the process used to confirm that analytical procedures employed for specific tests are suitable for their intended use. It is an integral part of any good analytical practice. Methods need to be validated or revalidated [3]. There are articles in the literature validating analytical methods using HPLC for the quantification of ZPT in shampoos [2,4,5,6]. However, analytical methods by UV-VIS spectrophotometry are fast, simple and cheap [7]. The aim of this work was to develop and validate an analytical method to determine ZPT content in formulations of commercially available shampoos and compounded shampoos, evaluating the content and physicochemical characteristics of different shampoos.

2 MATERIALS AND METHODS

2.1 OBTAINMENT OF SHAMPOOS

To obtain the compounded formulation, it was used a glycerinated shampoo with pearlescent base, added to three surfactants; obtaining afterwards a placebo and a formulation containing zinc pyrithione at 1%, the sample was obtained at the Pharmacy School of the Federal University of Piauí. The industrialized shampoos were purchased in a commercial market in the city of Teresina - Piauí and the packaging labels were removed to conduct the tests. The samples were titled A, B, C and Compounded.

3 WORK STANDARDS

For the development and validation of the analytical method of shampoo it was used Zinc pyrithione (Fagron® lot k006.2 / 15/03/017 - content 48.0 - 50.0%) as the working standard.

3.1 REAGENTS

For the dilution of Zinc pyrithione it was used distillate water.

3.2 VALIDATION OF THE ANALYTICAL METHOD

The validation of the analytical method was performed following the parameters: Specificity and Selectivity, Linearity, Accuracy, Precision, and Robustness established by ANVISA in the RDC nº 166/2017, validation of analytical procedures: text and methodology (Complementary Guideline on Methodology dated 6 November 1996 Incorporated in November 2005) and Analytical procedures and methods validation for drugs and biologics (Food and Drug Administration Guidance for Industry) [8,9,10].

Analytical method validations the process of demonstrating that an analytical procedure is suitable for its intended purpose. The methodology and objective of the analytical procedures should be clearly defined and understood before initiating validation studies. This understanding is obtained from scientifically-based method development and optimization studies [10].

Specificity was verified through spectrophotometric scanning (Shimadzu® UV-1800 Spectrophotometer) using the standard sample, Zinc pyrithione 1% shampoo and the shampoo without Zinc Pyrithione.

Linearity was analyzed through the construction of an analytical curve using points 5.0; 9.0; 12.0; 18.0 and 24.0 $\mu g.mL^{-1}$ The analytical curves were made in triplicate in which the maximum wavelength suitable for quantification of zinc pyrithione was 239.0 nm, as evidenced by the graphical analysis of scanning spectra, in the zero order.

The Limits of Detection (LOD) and Quantification (LOQ) were determinate by means of calculations predefined by formulas, where LOD = SD x 3/IC and LOQ = SD x 10/IC, where SD is the standard deviation of the linear coefficients obtained with the three linearity curves and IC is the mean of the angular coefficients of the respective curves, according to RDC 166/2017 [8].

Precision analysis was performed through the repeatability precision (intra-run) where it was established in sixfold, contemplating the value of 100% of the formulation content, and the intermediate precision (inter-run) that was performed in a 24 hours interval, by different analysts.

The parameters accuracy were evaluated by the proximity of the obtained results regarding the true value, using values contemplating 100% of the content of the formulation, as

well as formulations with Zinc Pyrithione with values varying 20% for more and for less. The analysis was performed in triplicate.

For Robustness analysis, it was verified the sample stability at times: 0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5 hours and were altered from agitation to obtain the standard for reading the samples.

3.3 STATISTICAL ANALYSIS

This analysis was performed through the program Origin 8.0®, simple analysis of variance (ANOVA), or trough test t-Student ($p<0,05$).

3.4 PHYSICOCHEMICAL CHARACTERIZATION

Formulations were submitted to organoleptic characterization tests, content determination, pH, viscosity, foam index and conductivity. The standard characteristics of shampoos were used as parameters for analysis of the samples organoleptic characteristics. Formulation asp/ects such as uniformity, good presentation and texture were evaluated.

For content determination, a stock solution of each sample was obtained and then were carried out dilutions in triplicate to determine the zinc pyrithione content using a previously validated methodology of UV-Vis analytical quantification, the analytical curve was constructed in points 5.0, 9.0, 12.0, 18.0 and 24.0 $\mu g.mL^{-1}$.

The samples pH was measured by a potentiometer Bel W3B, in triplicate, at room temperature (25.0 ± 2.0 ºC) [11]. Viscosity was evaluated according to the Brazilian Pharmacopoeia, 5ed., in a digital rotational viscometer previously calibrated, in triplicate, at room temperature (25.0 ± 2.0 ºC), using spindle 2 (SP2), in Speeds 6, 12, 30 and 60 rpm [11].

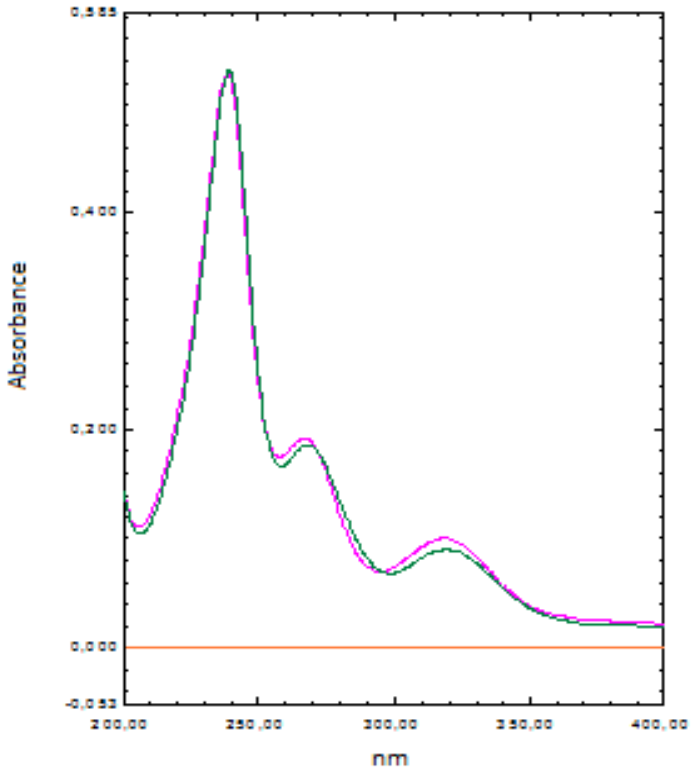
For determination of the foam index, 9 ml of the shampoo sample were added to 1 mL of distilled water in a medium test tube, which was shaken 30 times within a period of 30 seconds. The foam was measured with a ruler at times: 0, 5, 15 and 20 minutes.

Conductivity test was performed using the MB-11 Marte Conductivity Meter according to the Brazilian Pharmacopoeia 5ed. The samples were read in triplicate at 25°C [11].

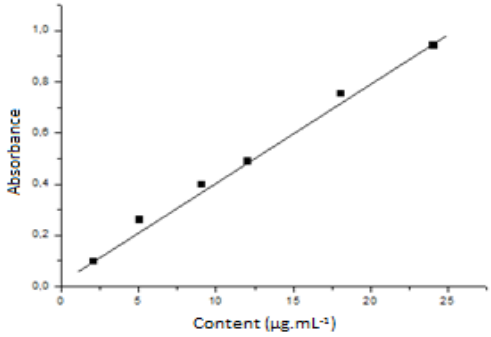
4 RESULTS AND DISCUSSION

It was observed linearity, where the correlation coefficient (r) presented a value equal to 0.99511, and the line equation was $y = 0.036606x + 0.075963$, as it can be observed in Fig.1. The method was also specific, without the presence of interferers with the formulation.

Figure 1: Analytical curve for determination of zinc pyrithioneate in pearlescent base shampoo at 239.2 nm



Footnote: Orange line: placebo; green line: standard, purple line: compounded formulation



Source: Research data

By observation of Table I it is possible to infer that the method showed to be accurate, the repeatability analysis obtained CV% between 3.71 and 3.91%, according to the recommended resolution. The intermediate accuracy study was also compatible with the Resolution where the value obtained for the CV% was between 3.17 and 3.79%. The results did not show statistically significant differences for $p < 0.05$.

Table I. Precision of zinc pyrithione by visible spectrofotometry

ASSAY		CONCENTRATION (µg.mL^{-1})	MEAN	SD	CV%
Repeatability (n=6)		12	11,70	0,23	1,95
Intermediate precision (n=6)	**Analist A 1st day**	12	11,47	0,27	2,32
	Analist B 1st day	12	11,38	0,25	1,34
	Analist A 2nd day	12	11,90	0,33	2,53
	Analist B 2nd day	12	11,75	0,41	3,47

Source: Research data

In Table II it is shown that the accuracy varied in a small amount, in relation to 100% of the asset, presenting 99.51% as the lower value and 100.73 the highest value, thus demonstrating the accuracy of the proposed method.

Table II. Accuracy expressed in percentage of Zinc Pyrithione by visible spectrophotometry

THEORETICAL CONCENTRATION (µg.mL^{-1})	MEAN EXPERIMENTAL CONCENTRATION (µg.mL^{-1}) (n=3)	ACCURACY %
9,6	9,55	99,51
12	12,09	100,73
14,4	14,44	100,28

Source: Research data

The method was considered robust within the parameters evaluated in Table III, where even with modifications of the stirring rate in the preparation of the solutions, content remained within specifications.

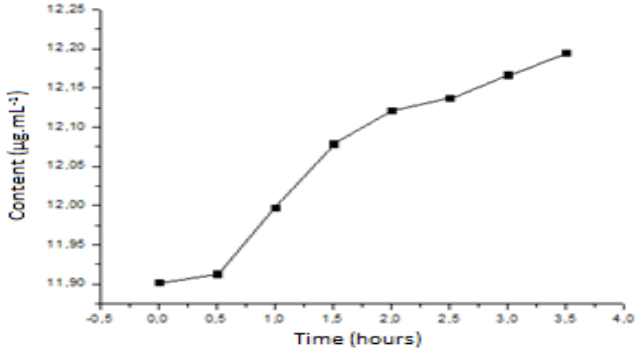
Table III. Robustness with stirring rate modification (manual and magnetical) by visible spectrofotometry

	RATE MODIFICATION			
THEORETICAL CONCENTRATION (µg.mL^{-1})	MANUAL CONCENTRATION (n=3)	CV%	MAGNETICAL CONCENTRATION (n=3)	CV%
9,6	9,58	0,95	9,54	0,96
12	12,19	0,41	12,10	0,60
14,4	14,50	0,56	14,52	0,29

Source: Research data

The method also presented as a positive parameter the robustness, where the solution for reading remained stable over the hours, as observed in Fig.2 and did not present statistically significant differences, considering a 95% confidence interval.

Figure 2. Stability of the solution used for reading at times: 0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5 hours at 239.2 nm



Source: Research data

The shampoos presented themselves uniforms, with a good appearance, which is very important for a cosmetic product, consistent texture and without phases separation.

Zinc pyrithione is an antifungal and antibacterial agent and it should be used topically in concentrations around 1-2% [12]. As can be observed in Table IV, none of the commercial samples analyzed presented a content range between 1 - 2%, in which brand B presented the lowest content and a value insufficient to promote antifungal action. However, brands A and C presented very close values and sufficient to promote action. The shampoo compounded at 1% presented content within the range to present antifungal action.

Table IV. Determination of zinc pyrithione content in different brands of shampoos and compounded shampoo

BRAND	CONTENT(%)
A	0,95±0,33
B	0,30±0,03
C	0,93±0,12
COMPOUNDED	1,02±0,02

Source: Research data

The pH and organoleptic characteristics of the product itself allows the observation of whether the raw materials are suffering degradation with storage. In addition, it should be noted that the scalp, due to dandruff, is suffering a physical aggression and if the pH is not ideal it may lead to other problems, such as irritation, causing discomfort to the patient, and therefore leading to interruption of the treatment. Thus, it is noted that the formulations pH is important to detect possible changes over the time, ensuring that the value is compatible with the formulation content and the application site, thus avoiding irritations [13]. The pH of the samples varied between 5.41 and 6.25, being within the values compatible with the scalp (**Table V**).

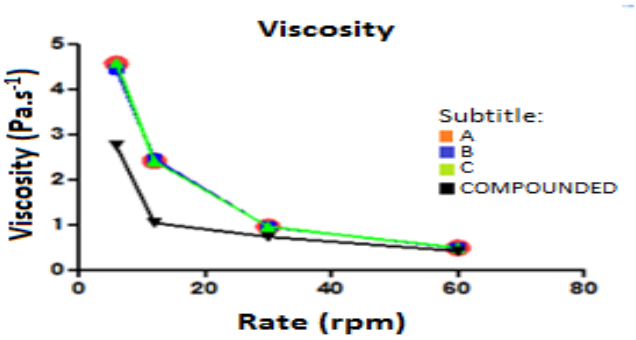
Table V. Determination of pH in different brands of shampoos

BRAND	pH
A	6,25±0,02
B	6,09±0,03
C	5,97±0,08
COMPOUNDED	5,41±0,07

Source: Research data

Viscosity showed a decrease with increases of the rotation speed, it can be observed in Fig.3 that the compounded shampoo presented a lower viscosity initially, but with an increase in rotation the samples had the same viscosity at the speed of 60 RPM. Commercial shampoos presented very close viscosities with increased rotation.

Figure 3. Graph of viscosity by the speed of rotation (RPM) of samples A, B, C and COMPOUNDED



Source: Research data

Foam content does not interfere with the physicochemical characteristics of the shampoo, it only characterizes a better foam formation, which pleases the consumer. Observing Table VI, it can be observed that the shampoo A presented a higher index of initial foam and persistent foam, shampoo C had the lowest initial foam index and also the lowest index of persistent foam.

Table VI. Determination of the foam index at the times, 0, 5, 15, 20 minutes

Time / Brand	0 min	5 min	15 min	20 min
A	10,5cm	9,1cm	9,1cm	9,1cm
B	8cm	7cm	6,5cm	6,5cm
C	7cm	5,3cm	5,3cm	5,3cm
COMPOUNDED	8,5cm	7cm	7cm	7cm

Abreviattions: min - minutes, cm - centimeters
Source: Research data

Conductivity differed significantly amongst the samples. As can be seen in Table VII, sample A showed the highest conductivity, followed by samples C, B and the compounded shampoo.

Table VII. Determination of conductivity (S) in different brands of shampoos

BRAND	S (Us.mL^{-1})
A	1131,67±7,57
B	553,73±9,52
C	852,07±27,12
COMPOUNDED	155,33±1,79

Source: Research data

5 CONCLUSION

According to the results the methodology proved itself to be specific, cheap, simple and fast. Thus, it can be used for the determination of zinc pyrithione in shampoos with a pearlescent base, supplying all analysed parameters and presenting reliability and consistency. In the characterization of the shampoos, one of the commercially available shampoos presented a zinc pyrithione content inferior to the contents specified in the literature to promote antifungal action. This shampoo may not promote the expected effect by the consumer. Thus, the evaluation of anti-dandruff shampoos based on ZPT are important for the final quality of the product.

ACKNOWLEDGMENTS

Pharmacy School of the Federal University of Piauí

REFERENCES

1. Chen G, Miao M, Hoptroff M, Fei X, Collins LZ, Jones A. Sensitive and simultaneous quantification of zinc pyrithione and climbazole deposition from anti-dandruff shampoos onto human scalp. *J Chromatogr B.* **1003,** 22-26 (2015)

2. Thomas KV. Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry. *J Chromatogr A.* **833,** 105-109 (1999).

3. Chaudhary A, Singh A, Verma P.K. Method development and validation of potent pyrimidine derivative by UV-VIS spectrophotometer. *Org Med Chem Lett.* **4-15,** (2014).

4. Nakajima K, Ohta M, Yazaki H, Nakazawa H. High-Performance Liquid Chromatographic Determination of Zinc Pyrithione in Antidandruff Shampoos Using On-Line Copper Chelate Formation. *J. Liq. Chromatogr.* **16,** 487-496 (1993).

5. Kondoh Y, Takano S. Determination of zinc pyrithione in cosmetic products by high-performance liquid chromatography with pre-labelling. *J Chromatogr A.* **408,** 255-262 (1987).

6. Gu YX, Wang QH, Zhou ZL, Lv Q, Mai, CH. Determination of zinc pyrithione in shampoos by HPLC and HPLC-MS/MS. *J Cosm Sci*. **65,** 265-276 (2013).

7. Shailaja B, Swarna MK, Afreen A, Kumar A. A rapid assay method development and validation for the estimation of eplerenone in tablets by uv spectrophotometry. *Int J Pharm Pharm Sci*. **7,** 327-330 (2015).

8. ANVISA. Agência Nacional de Vigilância Sanitária Resolução da Diretoria Colegiada - RDC nº 166 de 24 de julho de 2017; Brasília, 2017

9. European Medicines Agency; ICH Harmonised tripartite guideline - Validation of analytical procedures: text and methodology Q2 (R1); London, 2005.

10. U. S. Food and Drug Administration; Analytical procedures and methods validation for drugs and biologics Guidance for Industry; Rockville, 2015

11. ANVISA. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Farmacopeia brasileira. 5ª ed. Brasília (DF): Brasil, 2001.

12. Bellare J, Iyer R, Olly CI. A study on the conditioning effects of natural shampoos using the scanning electron microscope. *Int J Cosmet Sci*. **23,** 139-145 (2001).

13. Cunha AR, Silva RS, Chorilli M. Desenvolvimento e avaliação da estabilidade física de formulações de xampu anticaspa acrescidas ou não de extratos aquosos de hipérico, funcho e gengibre. *Rev Bras Farm*. **90,** 190-5 (2009).