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Analysis of ZnPtO in Anti-dandruff Shampoo by High-Performance Liquid Chromatography - *Photo Diode Array*

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ABSTRACT

ZnPtO is widely used as an active substance in anti-dandruff shampoo. Instead of describing its benefits, many articles have reported that ZnPtO can harm the environment and human health. Through the Food and Drugs Administration, Indonesia has been regulated to have a maximum limit of 2.0% for rinse-off hair products. The research aims to develop an accurate and reliable method to determine product ZnPtO level. ZnPtO was analyzed using High-Performance Liquid Chromatography (HPLC) with Photo Diode Array (PDA) at a wavelength of 257.9 nm. This research used a C18 column with dimensions of 250 x 4.6 nm and 5 µm in particle size. The mobile phase consisted of acetonitrile and a mixture of potassium dihydrogen phosphate solution and disodium EDTA at pH 4.0 (30:70). The column temperature was maintained at 40°C at a 1.0 ml/min flow rate. The results showed that ZnPtO was detected at a retention time of 7 minutes. The method's correlation coefficient and residual deviation were 0.999% and 0.65%, respectively. Method precision at 20, 100, and 160 µg/ml was 0.6694, 0.4511, and 0.4728%, respectively. Method accuracy at those levels was 98.3 to 100.9%. All validation parameters have fulfilled the qualification. ZnPtO levels contained in anti-dandruff shampoos were 0.0081%, 0.0040%, and 0.016%, respectively. The developed method has proven selective, accurate, and reliable. It can be used to control the quality and safety of anti-dandruff shampoo due to pre-market and post-market surveillance.

ZnPtO merupakan senyawa aktif dalam produk sampo anti ketombe. Meskipun memiliki banyak kegunaan, namun beberapa artikel ilmiah menyebutkan adanya dampak penggunaan senyawa ini baik terhadap lingkungan maupun terhadap hewan uji. Sebagai zat aktif dalam produk anti ketombe, Indonesia melalui Badan Pengawas Obat dan Makanan (BPOM) telah mengatur batas aman kandungan ZnPtO maksimal sebesar 2,0% dalam produk sediaan rambut bilas. Penelitian ini dilakukan untuk mengembangkan metode analisis yang akurat dan handal untuk menguji kandungan senyawa ZnPtO di dalam produk sampo anti ketombe. ZnPtO dapat dianalisis menggunakan instrumen Kromatografi Cair Kinerja Tinggi (KCKT) menggunakan detektor Photo Diode Array (PDA) pada panjang gelombang 275,9 nm dengan kolom C18 (250 x 4,6 mm dengan ukuran partikel 5 µm) menggunakan fase gerak asetonitril dan campuran larutan larutan kalium dihidrogen fosfat – dinatrium EDTA pH 4,0 (30 :70). Suhu kolom dijaga pada 40°C dan laju alir 1,0 ml/menit. Hasil analisis menunjukkan baku ZnPtO terdeteksi pada waktu retensi sekitar 7 menit. Nilai koefisien korelasi (r) dan deviasi residual (Vx0) pada penetapan linieritas metode berturut-turut adalah 0,999% dan 0,65%. Presisi metode pada konsentrasi 20; 100; dan 160 µg/ml berturut – turut adalah 0,6694%; 0,4511% dan 0,4728%. Akurasi metode pada tiga

konsentrasi tersebut berada pada rentang 98,3 – 100,9%. Seluruh parameter validasi telah memenuhi syarat. Hasil uji kadar ZnPtO dalam sampel sampo anti ketombe yaitu 0,0081%, 0,0040% dan 0,016%. Metode analisis yang dikembangkan terbukti selektif, akurat dan andal, sehingga dapat digunakan sebagai metode uji dalam rangka kontrol kualitas dan keamanan sebelum dan selama produk beredar.

Keywords: HPLC-PDA, method validation, Zinc Pyrithione
Kata Kunci: *KCKT – PDA, validasi metode, Zink Pirition*

1. Introduction

In everyday life, humans are inseparable from using cosmetics both for caring purposes, changing or improving appearance, and covering body odor (Sharma et al., 2018). According to the Food and Drug Authority Regulation No. 17 of 2022 concerning Amendments to the Food and Drug Authority Regulation No. 23 of 2019 concerning Technical Requirements for Cosmetic Ingredients, cosmetics are materials or products intended for use on the external parts of the human body such as the epidermis, hair, nails, lips, and external genital organs, or teeth and oral mucous membranes, especially for cleaning, perfuming, changing appearance, and/or improving body odor or protecting or taking care the body in good condition. (BPOM RI, 2022). One type of cosmetic that is widely used in daily life is shampoo.

Shampoo is the most common hair product used by the public. Shampoo cleans the oil/sebum, sweat, the dirt that sticks to the scalp, and other cosmetic products used for hair styling (George & Potlapati, 2021). In addition to cleaning the hair's dirt, shampoo also used to remove dandruff. Dandruff is excessive flaking of the scalp accompanied by the presence of fatty impurities, itching, and hair loss. Dandruff is caused by infection process with microorganisms, including *Malassezia furfur*, *Malassezia globosa,* and *Malassezia restricta*. (Das & Khubdikar, 2019) (Pertiwi et al., 2020).

The compound that is generally added in anti-dandruff shampoos is *zinc pyrithione* (ZnPtO) (Leong et al., 2020). This compound is an active ingredient with low irritation and sensitization potential in anti-dandruff. ZnPtO is an anti-fungal ingredient widely used in shampoos to treat seborrheic dermatitis and dandruff symptoms (Mangion et al., 2021). Although the risk of sensitization is low, cases of allergic dermatitis are still found due to skin contact with ZnPtO compounds (Mangion et al., 2021). Apart from being used in anti-dandruff shampoo formulations, ZnPtO can also be used in topical formulations to treat localized psoriasis.

ZnPtO ($ZnC_{10}H_8N_2O_2S_2$) is a coordination complex compound with a 2-valence zinc cation (Zn^{2+}) as central atom with two bound ligands, namely pyrithion anions (Kim et al., 2018). The molecular weight of this compound is 317.7 g/mol and its logP is 0.88. These properties make the ZnPtO molecule very *permeable* when applied to the skin. However, the permease properties of ZnPtO on the skin are limited because this compound has a low solubility in water. This makes ZnPtO ideal for use in shampoo formulations (Mangion et al., 2021). As an active ingredient for anti-dandruff shampoos, ZnPtO are often combined with other compounds, such as climbazole, to provide anti-dandruff efficacy with more significant benefits (Turner et al., 2013).

Even though ZnPtO has good effectiveness as an anti-fungal in anti-dandruff shampoo, contact or ingestion of this compound to a certain extent level can have negative impact ; where according to the *Scientific Committee on Consumer Safety,* above 2000 mg/kg of

ZnPtO level can cause acute dermal toxicity (SCCS, 2020). In laboratory studies, ZnPtO compounds are reported to trigger various responses, such as DNA damage in skin cells (Park et al., 2020). ZnPtO, as an active ingredient in anti-dandruff shampoo, has the potential to adhere on the scalp, which can at least cause mild irritation even if shampoo is used by rinsing. Determination of ZnPtO levels in the scalp showed that shampoos containing a combination of ZnPtO and climbazole gave more ZnPtO deposits than shampoos using only a single ZnPtO (Chen et al., 2015).

In the Indonesian Food and Drug Authority (BPOM) Regulation No. 17 of 2022, the requirements for ZnPtO are listed in Appendix 1 of the List of Ingredients Allowed for Use in Cosmetics with Restrictions and Requirements for Use. The content of ZnPtO in the hair rinse off product as anti-dandruff should not be more than 2.0% (BPOM RI, 2022). To determine the quality and safety of anti-dandruff shampoo, it is necessary to establish an analytical method to detect the product's ZnPtO level. Currently, the analytical method available as.

BPOM's *in-house method* for testing the ZnPtO content in anti-dandruff shampoo is a manual titration method with a long processing time, a complicated preparation process, and requires a large amount of reagents. This method is considered ineffective and inefficient for monitoring many products circulated in the market. In addition, the manual titration method requires high accuracy from each laboratory staff to determine the endpoint of the titration, allowing for potential errors in concluding the test results. To minimize this potency, it is necessary to develop a new analytical method to determine the ZnPtO content in anti-dandruff shampoo quickly, precisely, and accurately.

Previous research reported that ZnPtO content in anti-dandruff shampoo can be determined using complex and potentiometric titration methods. The complexometric test procedure uses several reagents, including hydrochloric acid and hydrogen peroxide. Ammonia and aqueous solution are added for pH adjustment to reach a pH of 10. EDTA solution (0.01 M) is used as a titrant with eriochrome black T as the indicator. The endpoint is reached if a color changes from purple to blue. In potentiometric testing, the titrant solution used is iodine solution (0.05 M), where the endpoint observation is carried out using a platinum electrode (Egurrola et al., 2021).. The use of complexometric and potentiometric titration methods is less sensitive and allows for errors in determining the endpoint of the titration.

Several other studies have used high-performance liquid chromatography (HPLC) techniques to analyze ZnPtO content. The analysis technique generally focuses on preparing to obtain ZnPtO compounds with good solubility before being analyzed on KCKT. The method developed uses a normal phase HPLC system using Porasil column and was reported to provide good precision and accuracy values. In that study, the preparation was carried out through a liquid liquid extraction technique using 10 mM copper sulfate solution and methylene chloride solution (1:1, v/v), and 5 ml of isopropanol in 2 liters of methylene chloride as mobile phase. (Fenn & Alexander, 1988).

Another study mentioned that used of RP-18 column with a mobile phase of acetonitrile water containing phosphate-buffered and *ethylenediaminetetraacetic acid* (EDTA) can determine the level of ZnPtO. Sample preparation was carried out using a methanol-water solvent mixture containing acetic acid and EDTA to increase the solubility of ZnPtO. The detection limit value obtained of 2 ng calculated based on the signal per noise ratio (Gagliardi et al., 1998).

In another study, the application of reversed-phase HPLC preliminary with the extraction process using dichloromethane and methanol was reported. The HPLC analysis used oxalic acid/EDTA in water (pH 4) and acetonitrile as mobile phases (Mildau, 2018).

The procedure to form ZnPtO complex compounds as copper complex compounds was also reported. The procedure carried out by adding copper sulfate solution and extraction using chloroform, and then analyzed uisng Reversed-phase HPLC. (Nakajima et al., 1990).

In the LC-MS/MS analysis of ZnPtO, sample preparation was reported carried out by dissolving the sample in a chloroform-methanol mixture (2:1, v/v). (Kim et al., 2018).

In other LC-MS/MS analysis of ZnPtO, samples are prepared by first washing them with water to remove surfactants and water-soluble impurities, then ultrasonically extracting them with acetonitrile-methanol (Gu et al., 2014). (Gu et al., 2014).

A reversed-phase HPLC technique with simple preparation technique has also been developed, which uses methanol as a solvent and provides a method LOD value of 4.10 µg/ml (Kachchhi et al., 2020).

Another analytical methods for testing ZnPtO content in shampoo that have been reported generally have complexity regarding sample preparation procedures, where the solvent and mobile phase combine several reagents. This study aims to find a more straightforward and faster preparation and analysis procedure of ZnPtO in anti-dandruff shampoo through chelate formation using EDTA solution so that it can be used as quality control and safety of anti-dandruff shampoo products before the products are circulated in the market and to facilitate BPOM as a regulator in the testing process in the context of supervision when the product circulating on the market to ensure the safety and quality of the product.

2. Methodology

2.1.Reagents and materials

The zinc pyrithione standard was obtained from the Center for National Quality Control Laboratory of Drugs and Food (PPPOMN BPOM). All reagents were used directly without further purification. Potassium dihydrogen phosphate (KH_2PO_4) analysis *grade* and *ethylenediaminetetraacetic acid disodium salt dihydrate* ($Na_2EDTA.2H_2O$) *reagent grade* from Sigma Aldrich, dimethyl sulfoxide (DMSO) analysis grade; methanol HPLC grade; orthophosphoric acid analysis grade from Merck and deionized water (18.2 MΩ cm at 25° C),– PTFE membrane filters of 0.45 µm were used to separate the target analyte from the interfering matrix.

2.2.Equipment

High-Performance Liquid Chromatography system was a Waters Alliance e2695 with an automatic injection system, pump, automatic sampler, thermal compartment, and a *Photo Diode Array* detector (2998 PDA). *Empower* software was used for system control, data processing, and collection. An octadecylsilane (C18) column with dimensions of 250 mm x 4.6 mm and a particle size of 5 µm was used for the separation process. The column temperature was maintained at 40° C during the analysis; the flow rate was 1.0 ml/min with an injection volume of 20 µl.

2.3.Solution Preparation

Preparation of Solvent and Mobile Phase. The solvents used were dimethyl sulfoxide (DMSO) as first solvent and HPLC grade methanol as second solvent. The mobile phase consisted of HPLC grade methanol (**A**) and a mixture of potassium dihydrogen phosphate

10 mM - sodium EDTA 25 mM pH 4.0 solution (**B**) with the ratio of A and B being 30 : 70. The pH of solution **B** was adjusted to 4.0 by the addition of orthophosphoric acid solution.

Preparation of Standard Solution. Zinc pyrithione standard stock solution was prepared at a concentration of 2000 µg/ml using first solvent in a volumetric flask. Series standard solutions were prepared by pipetting 1.0 ml, 2.0 ml, 4.0 ml, 5.0 ml, 7.0 ml, and 8.0 ml of the standard stock solution into separate 20 ml-flasks and diluted to volume with the first solvent. Furthermore, each solution was diluted with second solvent to obtain serial standard solutions with concentrations of 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, 140 µg/ml and 160 µg/ml, respectively.

Sample Preparation. Samples of cosmetic hair rinse off product (shampoo) were obtained from the samples circulated on the market. A 0.5 gram of sample was dissolved and diluted with first solvent in a 20-ml volumetric flask. Then, 1.0 ml of the sample solution was pipetted and diluted with second solvent in a 5-ml flask.

2.4.Method Validation

The method's selectivity was carried out by injecting the ZnPtO standard solution and methylisothiazolinone standard simultaneously, and then the resolution values of the two standard peaks were observed. Linearity, precision, and accuracy were analyzed using sample solution spiked with a standard solution so that the final concentration of solution equal to the concentration of serial standard solution. A serial standard calibration curve was created by comparing the concentration of ZnPtO in the solution (x-axis) with the area of ZnPtO obtained from the analysis (y-axis). Precision was established by analyzing samples spiked with known concentration of ZnPtO standard, and the percent repeatability was calculated. Accuracy was determined by calculating the percent recovery of the ZnPtO standard added to the sample. A standard solution of methylisothiazolinone with a concentration of 100 µg/ml was used for method selectivity analysis. The limit of quantitation was established through dilution of the sample spiked with ZnPtO standard solution so that the precision and accuracy of ZnPtO response met the requirements. (AOAC Internacional, 2023).

3. Results and Discussion

3.1.Validation of Analysis Methods

Methylisothiazolinone standard solution was used as a selectivity standard solution to develop this analysis method. Methylisothiazolinone was used as a selectivity standard because this compound has a similar wavelength as the wavelength of ZnPtO when analyzed with a *Photo Diode Array* (PDA) detector (Pham Ngoc Thuy et al., 2021). This compound is also commonly used as a preservative in cosmetic anti-dandruff shampoo products containing ZnPtO compounds (Tomás et al., 2020). Figure 1 shows the results of the selectivity analysis of the analysis method.

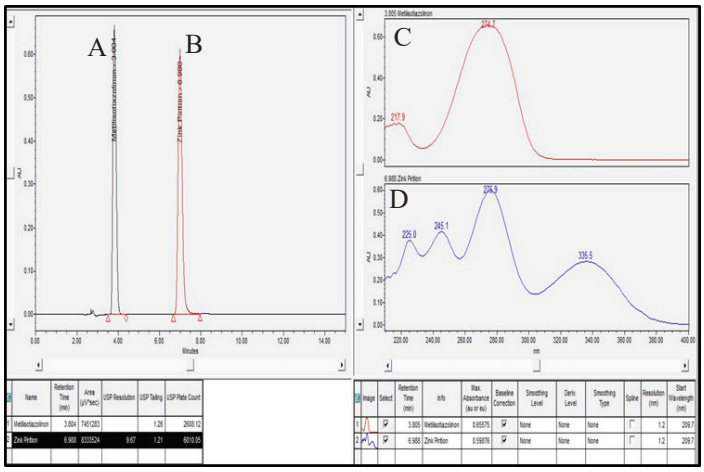

Figure 1. Chromatograms of (A) ZnPtO; (B) Methylisothiazolinone and PDA spectra of (C) Methylisothiazolinone ; (D) ZnPtO

Linearity, precision, and accuracy were analyzed by adding a certain amount of ZnPtO standard solution with concentrations of 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, 140 µg/ml, and 160 µg/ml to the sample. The concentration range selected in the validation method was adjusted to the levels of ZnPtO compounds commonly contained in circulating samples after orientation process of several samples and and also adjusted to the current regulations, where the level of ZnPtO in shampoo is not more than 2.0%. The concentration range above reflects the levels of ZnPtO contained in the shampoo of 0.4%, 0.8%, 1.6%, 2%, 2.8%, and 3.2%. With the selection of these concentration ranges, the analytical method is expected can be used for testing the ZnPtO content in anti-dandruff shampoo at low and high concentration conditions. Table 1 shows the solution codes used to determine the method's linearity, precision, and accuracy.

Table 1. Solutions for Determination of Linearity, Precision, and Accuracy

Validation Parameters	Solution Concentration (µg/ml)*					
	20	***40***	***80***	***100***	***140***	***160***
Linearity	P	Q	R	S	T	U
Precision	P	-	-	S	-	U
Accuracy	P	-	-	S	-	U

*Linearity was achieved by making two solutions for each concentration level (duplo), while precision and accuracy were achieved triple. Solutions were made 1x for all validation parameter determinations.

From the linearity analysis, the correlation coefficient (r) value obtained is 0.9999, and the residual deviation value (V_{x0}) is 0.65%. Based on the literature, the requirements for the value of the correlation coefficient and residual deviation (V_{x0}) are more than 0.999 and less than 5%, respectively (Indrayanto, 2018). While other literature states that the requirements for the correlation coefficient (r) and residual deviation (V_{x0}) are more than and equal to (≥) 0.995 and less than (<) 5% (Yuwono & Indrayanto, 2005). So that the linearity parameters of this validation method has meet the requirements ~~set~~. The calibration curve of ZnPtO Standard and series of ZnPtO concentration in spiked sample for linearity analysis can be seen in Figure 2.

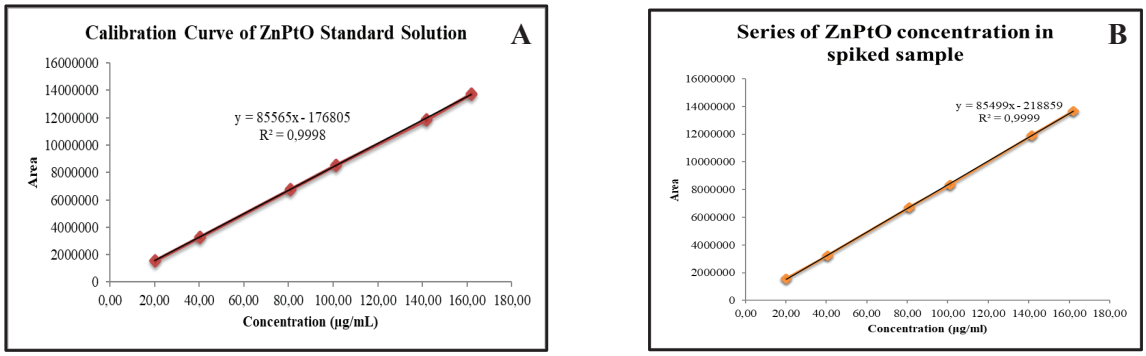

Figure 2. Calibration curve of ZnPtO standard (A); and series of ZnPtO concentration in spiked sample (B)

The method repeatability is expressed as a percentage of *Relative Standard Deviation* (%RSD). The %RSD value is a value that indicates the precision/repeatability of several data in a series test. The greater the %RSD value indicates that the precision/repeatability method is poor. This means that the method cannot be used for testing because one of the validation parameters does not meet the requirements stated in the *Official Methods of Analysis of AOAC International* (AOAC International, 2023). Measurement of the %RSD value is one of the validation parameters that must be measured to prove that the analytical method developed is robust with a good repeatability. The %RSD value in determining the method's precision was obtained as 0.6694%, 0.4511%, and 0.4728% at three different concentration levels. Meanwhile, the recoveries (%recoveries) in determining the method's accuracy were obtained in the range of 99.4 - 100.9%, 98.3 - 98.9%, and 99.3 - 100.0% for the three injected concentration levels. The limit of quantitation (LoQ) value obtained in this study was 0.23 µg/ml.

Table 2. Results of Linearity, Precision, and Accuracy Determination

Validation Parameters	Requirements	Results	Concentration (µg/ml)
Linearity	r < 0,995 V_{x0} <5%	r: 0,999 V_{x0}: 0.65%	20 - 160
Precision	≤ 7,3 ≤5,3 ≤5,3	0,6694% 0,4511% 0,47288%	20 100 160
Accuracy	80 - 110 % 90 - 107 % 90 - 107 %	99,4 - 100,9 % 98,3 - 98,9 % 99,3 - 100,0%	20 100 160

3.2.Analysis Result of ZnPtO Content in Samples

This developed analysis method for determining of ZnPtO content using a high-performance liquid chromatography (HPLC) instrument was carried out through chelate formation with Na_2EDTA compound. ZnPtO contained in the sample reacts with the mobile phase of the EDTA solution to form chelates according to the following reaction:

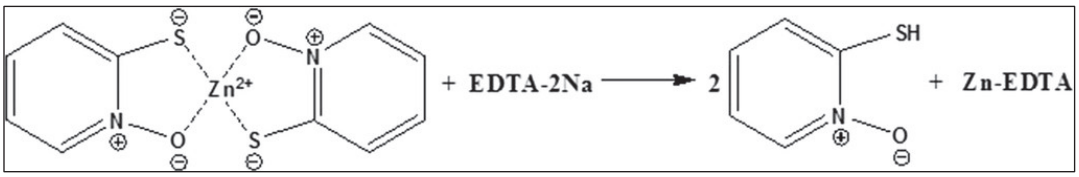

Figure 3. ZnPtO reaction with EDTA

EDTA solution will chelate the Zinc cation on ZnPtO so that it decomposes into pyrothione anion (Kim et al., 2018). With the formation of the Zn-EDTA chelate, ZnPtO compounds can be analyzed quickly using a HPLC instrument.

The sample preparation process in the developed analytical method is relatively simple because it is only carried out by dissolving the sample within a short analysis time.

The validation results of the method have proven its validity so that it can be used to analyze the active substance content of ZnPtO in 3 (three) anti-dandruff shampoo samples on the market.

The analysis results on several shampoo samples obtained randomly from the market are shown in Table 3.

Table 3. Analysis result of zinc pyrithione content in anti-dandruff shampoo

Sample Name	ZnPtO area	*Average Area of ZnPtO*	*ZnPtO concentration (µg/ml)*	*ZnPtO concentration (%)*
Sample X	6732381			
	7009619	6791789	81,44	0,0081
	6633366			
Sample Y	3227070			
	3240873	3227596	39,78	0,0040
	3214845			
Sample Z	13681325			
	13553106	13607980	161,10	0,016
	13589509			

Based on the test results of 3 (three) samples, the ZnPtO content in each sample was 0.0081%, 0.0040%, and 0.016%, respectively. These results indicate that all samples tested are still below the specified requirement of 2.0% (BPOM RI, 2022).

The developed analytical method can be used to analyze anti-dandruff shampoo samples circulating on the market. However, with the diverse matrix composition of anti-dandruff shampoos circulated on the market, it is necessary to apply the method to several other types of anti-dandruff samples. Thus, more representative test data regarding analytical methods for monitoring shampoo products on the market will be obtained.

4. Conclusion

The analytical method for determining the level of ZnPtO in anti-dandruff shampoo developed using the High-Performance Liquid Chromatography (HPLC) instrument has met all the validation parameters requirements so that the method can be used to analyze the ZnPtO content in anti-dandruff shampoo, both for quality control and product safety before and during the product circulating on the market due to guarantee public protection against

shampoo products that do not meet the requirements. Preparation of ZnPtO standard and samples using the developed method is more straightforward because it only goes through a dissolution process using a simple solvent with analytical conditions that are also relatively easy but can be applied to various anti-dandruff shampoo sample matrices.

Reference

AOAC Internacional. (2023). Appendix K: Guidelines for Dietary Supplements and Botanicals - Part I AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. In Official Method of Analysis of AOAC International - Appendix K Guidelines for Dietary Supplements and Botanicals (Issue 22). https://doi.org/10.1093/9780197610145.005.011

BPOM RI. (2022). Peraturan Badan Pengawas Obat Dan Makanan Nomor 17 Tahun 2022 Tentang Perubahan Atas Peraturan Badan Pengawas Obat Dan Makanan Nomor 23 Tahun 2019 Tentang Persyaratan Teknis Bahan Kosmetika Dengan. Bpom RI, 11, 1–16.

Chen, G., Miao, M., Hoptroff, M., Fei, X., Collins, L. Z., Jones, A., & Janssen, H. G. (2015). Sensitive and simultaneous quantification of zinc pyrithione and climbazole deposition from anti-dandruff shampoos onto the human scalp. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1003, 22–26. https://doi.org/10.1016/j.jchromb.2015.09.009

Das, S., & Khubdikar, K. (2019). A simple and facile spectrophotometric tool for quantification of zinc pyrithione (ZPT) in suspension. Chemical Data Collections, 19, 100175. https://doi.org/10.1016/j.cdc.2018.100175

Egurrola, G. E., Mazabel, A. P., & García, J. (2021). Development and Validation of a Complexometric and Potentiometric Titration Method for the Quantitative Determination of Zinc Pyrithione in Shampoo. Journal of Analytical Methods in Chemistry, 2021, 0–4. https://doi.org/10.1155/2021/6661744

Fenn, R. J., & Alexander, M. T. (1988). Determination of zinc pyrithione in hair care products by normal phase liquid chromatography. Journal of Liquid Chromatography, 11(16), 3403–3413. https://doi.org/10.1080/01483918808082263

Gagliardi, L., Multari, G., Cavazzutti, G., De Orsi, D., & Tonelli, D. (1998). HPLC determination of ciclopirox, octopirox, and pyrithiones in pharmaceuticals and antidandruff preparations. Journal of Liquid Chromatography and Related Technologies, 21(15), 2365–2373. https://doi.org/10.1080/10826079808000544

George, N. M., & Potlapati, A. (2021). Shampoo, conditioner and hair washing. International Journal of Research in Dermatology, 8(1), 185. https://doi.org/10.18203/issn.2455-4529.intjresdermatol20214930

Gu, Y. X., Wang, Q. H., Zhou, Z. L., Lv, Q., & Mai, C. H. (2014). Determination of zinc pyrithione in shampoos by HPLC and HPLC-MS/MS. Journal of Cosmetic Science, 65(5), 265–276.

Indrayanto, G. (2018). Validation of Chromatographic Methods of Analysis: Application for Drugs That Derived From Herbs. In Profiles of Drug Substances, Excipients and Related Methodology (1st ed., Vol. 43). Elsevier Inc. https://doi.org/10.1016/bs.podrm.2018.01.003

Kachchhi, R. R., Patel, D. R., Patel, A. B., Patel, K. S., & Patel, S. C. (2020). Stability Indicating Chromatographic Method Development and Validation of Sertaconazole nitrate and Zinc pyrithione in Shampoo. 8(4), 2566–2585.

Kepala Badan Pengawas Obat dan Makanan (BPOM). (2023). Keputusan Kepala Badan Pengawas Obat dan Makanan Nomor 479 Tahun 2023 tentang Perubahan Bahan yang Diizinkan dalam Kosmetik. 1–243.

Kim, T. H., Jung, G. H., Lee, E. H., Park, H. R., Lee, J. K., & Kim, H. G. (2018). Development and validation of liquid chromatography–tandem mass spectrometry method for simultaneous determination of zinc pyrithione and pyrithione in shampoos. Acta Chromatographica, 30(3), 200–205. https://doi.org/10.1556/1326.2017.00294

Leong, C., Wang, J., Toi, M. J., Lam, Y. I., Goh, J. P. Z., Lee, M., & Dawson, T. L. (2020). Brief Report Effect of zinc pyrithione shampoo treatment on skin commensal Malassezia. 1–4. https://doi.org/10.1093/mmy/myaa068

Mangion, S. E., Holmes, A. M., & Roberts, M. S. (2021). Targeted delivery of zinc pyrithione to skin epithelia. International Journal of Molecular Sciences, 22(18), 1–30. https://doi.org/10.3390/ijms22189730

Mildau, G. (2018). General Review of Official Methods of Analysis of Cosmetics. In Analysis of Cosmetic Products: Second Edition (Second Edi). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63508-2.00004-7

Nakajima, K., Yasuda, T., & Nakazawa, H. (1990). High-performance liquid chromatographic determination of zinc pyrithione in antidandruff preparations based on copper chelate formation. Journal of Chromatography A, 502(C), 379–384. https://doi.org/10.1016/S0021-9673(01)89602-3

Park, C. Y., Moon, S., Baek, S. H., Kim, M. W., Roh, J., Sung, J., & Park, T. J. (2020). Development of Detection Methods for Zinc Pyrithione in Polypropylene via Simple Extraction Methods for Quality Control. Biochip Journal, 14(2), 211–217. https://doi.org/10.1007/s13206-020-4210-7

Pertiwi, O. N., Aryani, R., Cahya, G., & Darma, E. (2020). Kajian Efektivitas Penggunaan Zinc Pyrithione dalam Sediaan Sampo Antiketombe. Prosiding Farmasi, 2(2), 861–865.

Pham Ngoc Thuy, V., Hung, T. V., Thang, P. N. T., Dang-Bao, T., & Anh, T. T. K. (2021). Determination of Methylisothiazolinone and Methylchloroisothiazolinone in personal care products by HPLC-DAD. IOP Conference Series: Earth and Environmental Science, 947(1). https://doi.org/10.1088/1755-1315/947/1/012022

SCCS. (2020). Opinion on Zinc Pyrithione (ZPT) - (CAS No 13463-41-7) - Submission III - Scientific Committee on Consumer Safety. 13463. https://health.ec.europa.eu/publications/zinc-pyrithione-zpt-submission-iii_en

Sharma, G. K., Gadiya, J., & Dhanawat, M. (2018). Textbook of Cosmetic Formulations (Pothi), ISBN: 9781365355912. May.

Tomás, M., Agonia, A. S., Borges, L., de Oliveira, A. P., & de Oliveira, R. P. (2020). Isothiazolinones quantification in shampoo matrices: A matter of method optimization or stability driven by interactions? Cosmetics, 7(1). https://doi.org/10.3390/cosmetics7010004

Turner, G. A., Matheson, J. R., Li, G. Z., Fei, X. Q., Zhu, D., & Baines, F. L. (2013). Enhanced efficacy and sensory properties of an anti-dandruff shampoo containing zinc pyrithione and climbazole. International Journal of Cosmetic Science, 35(1), 78–83. https://doi.org/10.1111/ics.12007

Yuwono, M., & Indrayanto, G. (2005). Validation of Chromatographic Methods of Analysis. Profiles of Drug Substances, Excipients and Related Methodology, 32(05), 241–260. https://doi.org/10.1016/S0099-5428(05)32009-0