ISSN 3031-0911

Determination of Hydroquinone Content in Whitening Toner Sold in Online Market Place X by Reverse Phase High Performance Liquid Chromatography (HPLC)

Stevani Hana Marumata[1*], Melania Perwitasari[2], Intan Kurnia Putri[3], Maya Uzia Beandrade[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia

[*]Correspondence author: vanymarumata@gmail.com

Abstract

Hydroquinone is often misused in cosmetic formulations as a whitener. According to the Badan Pengawas Obat dan Makanan/BPOM (Food and Drug Administration of Indonesia) regulation No. 23 of 2019, the content of hazardous substances and dyes such as hydroquinone is prohibited for use in cosmetics except for artificial nail preparations. This study aimed to determine the hydroquinone content in facial whitening toners sold in the X online marketplace using high-performance liquid chromatography (HPLC). This research is descriptive and non-experimental, and 15 toner samples were taken at online marketplace X with a purposive sampling method. Qualitative analysis was carried out by organoleptic and colour tests. Quantitative analysis on whitening toner was performed using the reverse phase HPLC method. The detection wavelength of 295 nm was applied, and a linear regression formula of y = 42.799x – 8.2248 with r = 0.9996 was obtained. Analytical method validation has been successfully executed and resulted in the resolution value of Rs 4.042, recovery percentage of 96.99%, RSD value of 0.801%, LOD and LOQ values of 0.7 ppm and 33.45 ppm, respectively. Hence, it can be stated that the RP-HPLC method was specific, accurate, and sensitive. The quantitative analysis test results showed that 8 out of 15 samples contained hydroquinone with an average concentration in sample T2 obtained 0.36499%, T4 0.35248%, T6 0.01253%, T7 0.35271%, T8 0.00371%, T9 0.00516%, T12 0.00272% and T13 0.00272%. According to the research data, it can be concluded that the hydroquinone content used in the whitening toner samples does not meet the BPOM requirements.

Keywords: *Bleach Toner ; HPLC ; Hydroquinone ; Validation*

Introduction

Based on research conducted by BPOM in 2019, it was found that the prevalence of 29% of cosmetic products contained the dangerous ingredient hydroquinone. From June 2020 to September 2021, a 25% prevalence of cosmetic products containing the hazardous ingredient hydroquinone was found. Based on this data, it has been proven that hydroquinone use in skin whitening cosmetics is still widely practised and widely circulated in society even though there are regulations prohibiting hydroquinone (BPOM, 2022). In the 2019 Food and Drug Supervisory Agency (BPOM) regulations regarding cosmetics containing dangerous substances and prohibited dyes, 0% hydroquinone is used in cosmetic preparations. Hydroquinone can only be used for nails at a level of 0.02% and hair dye at a maximum level of 0.3% because it can cause harmful health. This is because the side effects of using hydroquinone on the skin are irritation, the skin becoming red/erythema and a burning feeling. This effect will occur if hydroquinone is used in high concentrations. Hydroquinone concentrations above 0% for extended or continuous use will cause contact leukoderma and exogenous ochronosis. In addition, there is evidence that hydroquinone can cause cancer in mice after oral administration and can cause ochronosis (dark skin and black spots) when applied topically to the skin (Ruiyi *et al*., 2018).

Based on previous research, checking hydroquinone levels in cosmetic products is predominantly done on whitening creams and lotions. However, it has never been carried out on toners in the Bekasi district and Bekasi city. So, the researchers were interested in analyzing hydroquinone levels using the reverse phase High-Performance Liquid Chromatography (HPLC) method and bleach toner samples circulating in online marketplace X in the Bekasi district and Bekasi city. This research is expected to provide information regarding the presence or absence of hydroquinone content in whitening toner products.

Methods

Design

ISSN 3031-0911

This study uses a descriptive, non-experimental research design using purposive sampling. This research was conducted at the Pharmaceutical Chemistry Laboratory, Mitra Keluarga Bekasi College of Health Sciences in January-April 2023 with a population of all cosmetic shops in Bekasi city and Bekasi district in the X online marketplace shop.

Qualitative Analysis

Samples consisting of 15 toner products sold in the online marketplace X in the Bekasi city and Bekasi district were subjected to organoleptic testing, namely testing the colour, texture and aroma of the toner (Sari *et al*., 2021). Each toner sample was measured as much as 5 mL and then stored in a test tube. Then, 4 drops of Benedict were added, and colour changes were observed. Suppose there is a change in colour to brick red. The positive sample containing hydroquinone compounds will be followed up with a quantitative test using Reversed-Phase High-Performance Liquid Chromatography (HPLC).

Quantitative Analysis

The first preparation of the mobile phase was prepared with a mixture of 100 mL of methanol and distilled water (55:45), then filtered using a 0.45 µm millipore filter membrane and de-aired with an ultrasonicator for 10 minutes. Make a standard solution of 100 ppm, namely 5 mg of pure hydroquinone, dissolve it in 50 mL of mobile phase, then shake the solution until homogeneous. Make a standard calibration curve for hydroquinone, namely taking 3 mL, 3.5 mL, 4.5 mL, 6 mL and 8 mL of 100 ppm hydroquinone standard solution and dissolving in 25 mL of mobile phase and then shaking until homogeneous and concentrations of 12 ppm, 14 ppm, 18 ppm, 24 ppm and 32 ppm were obtained. Determination of the maximum wavelength is carried out by scanning a 20 ppm hydroquinone standard liquid, which is included in a cuvette using a blank solution, namely methanol p.a and the absorbance is measured at a wavelength of 200-400 nm so that the spectrophotometer will display maximum wavelength data by producing the very maximum absorbance value (Alqarni *et al*., 2021).

Validation Method

The specificity test was carried out by putting 1 mL of sample into a 10 mL volumetric flask, adding 1.4 mL of a 100 ppm standard solution, and entering the mobile phase up to the mark to make a volume with a final concentration of 14 ppm then shaking until homogeneous. Filter the solution with a 0.45 µm filter membrane into the vial and then in the sonicator for 10 minutes to remove bubbles and inject it into the HPLC instrument. Note each peak's resolution value (Rs) to see perfect separation. The accuracy test was carried out by inserting 1 mL of sample into 3 10 mL volumetric flasks and adding the mobile phase up to the boundary mark to make replications 1, 2 and 3. 1 mL of sample was taken and transferred into 9 10 mL volumetric flasks containing 1.4 mL; 1.8 mL and 2.4 mL of 100 ppm hydroquinone standard solution. A mobile phase was added to make final concentrations of 14 ppm, 18 ppm and 24 ppm. To get the % recovery, calculate the concentration using HPLC (Rahmayuni et al., 2018). The linearity test was obtained at five hydroquinone concentration levels (12 ppm, 14 ppm, 18 ppm, 24 ppm and 32 ppm), and then the area was plotted in the linear regression equation. The precision test was carried out by preparing a hydroquinone solution with a concentration of 18 ppm, measuring the area at the hydroquinone's retention time, and repeating the measurement 6 times. The standard deviation value and the coefficient of variation can be determined from the data obtained. The range test is determined from the lowest and highest concentrations of the hydroquinone standard series, which still meet the parameters of linearity, accuracy and precision (Saraswati, 2022). The limit of detection (LOD) and limit of quantification (LOQ) use a sample of the linearity parameters and are then calculated using the formula. This value is calculated from the standard deviation (SD) of the response and the slope of the curve (S) using the equation: LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), where SD is the standard deviation of the detector response and S: the slope of the calibration curve. The system suitability test was carried out with a standard mixed solution (18 ppm) injected as much as 20 µl into the HPLC with the selected analysis conditions. Six replications were carried out to ensure a constant peak area. The injection results were recorded, and the coefficient of variation (%CV) was calculated. The % CV value must be $\leq$ 2%. The system suitability test must meet resolution parameters, precision system determination, asymmetric factors, column efficiency, and capacity (Rahmayuni *et al.*, 2018).

Analysis of Hydroquinone Levels

The toner samples used were from various brands and purchased from the online marketplace X. A liquid sample (toner) was measured 1 mL in a beaker glass, and 10 mL of mobile phase was added and mixed until homogeneous. The solution is then filtered using a filter membrane (Alqarni *et al*., 2021). Hydroquinone levels, which met the validation

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parameters, were determined using HPLC. The HPLC system was set at a 1 mL/minute flow rate. The mobile phase used 55 mL methanol and 45 mL distilled water, and the stationary phase used a C18 analysis column. A total of 20 µL of sample was put in a vial, then analyzed for levels using HPLC. The results of the HPLC area obtained at each concentration are plotted into linear regression (Zukepli *et al*., 2015).

Results and Discussions

Qualitative Analysis

Organoleptic tests can be carried out using sensory organs such as smell, appearance, taste and texture. Organoleptic tests determine the odour, appearance, aroma and texture of samples. From the results of **Table 1**, as many as 7 samples (47%) had a pungent odour, 5 samples (33%) had a fragrant aroma, and 3 samples (20%) had no aroma. This test is carried out to determine the presence of hydroxyl groups responsible for hydroquinone's oxidation-reduction reaction, which reacts with Benedict's reagent to form a brick-red colour. From Table 1, there are 2 samples (13%) which changed colour to brick red, which is thought to contain hydroquinone, 6 samples (40%) which specifically changed from their original colour, and 7 samples (47%) which changed due to the influence of the colour of the reagent benedict.

Table 1. Color Test Results for Bleach Toner Samples with Benedict's Reagent

Sample Code	Reagents	Before Testing	After Testing	Result
Control (+)		Colorless	Brick Red	+
Control (-)		Light Yellow	Light blue	-
T1		Bright yellow	Light blue	-
T2		Yellow	Yellow Greenish	+
T3		Light Brown	Green	-
T4		Dark Brown	Yellow Greenish	+
T5		Pink	Light Brown	-
T6		Light Brown	Brick Red	+
T7	Benedict	Dark Yellow	Brick Red	+
T8		Light Blue	Greenish Brown	+
T9		Pink	Yellow Greenish	+
T10		Light Yellow	Light Blue	-
T11		Light brown	Light Purple	-
T12		White	Yellow Purplish	+
T13		Light Brown	Dark Brown	+
T14		Colorless	Light Blue	-
T15		Colorless	Yellow Greenish	-

Quantitative Analysis

Determination of the Maximum Wavelength of Hydroquinone

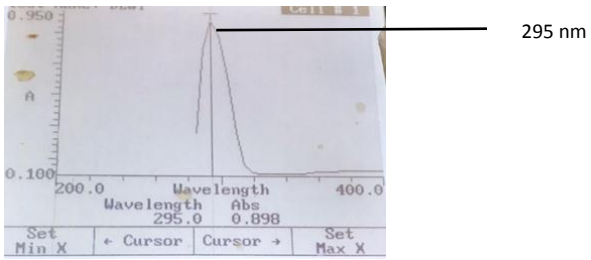

Figure 1. Maximum Wavelength of Hydroquinone with Methanol Solvent

Based on **Figure 1**, the maximum absorbance of hydroquinone is 0.898 with a wavelength of 295 nm obtained from the UV-Vis Spectrophotometer instrument.

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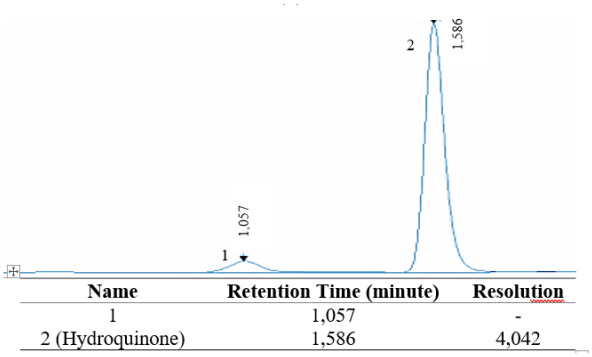
Validation Method

Specificity

The results obtained from the specificity test are separation or Rs of 4.042 with the chromatogram can be seen in the **Figure 2.**



Name	Retention Time (minute)	Resolution
1	1,057	-
2 (Hydroquinone)	1,586	4,042

Figure 2. Hydroquinone Specificity Test Value with Reversed Phase HPLC.

Accuracy

The results of accuracy testing with three concentrations have an average recovery value of 96.99% which can be seen in **table 2.**

Table 2. Accuracy Test Values for Hydroquinone with Reversed Phase HPLC

Concentration (ppm)	Rep	Concentration (ppm)			% *Recovery*	Average ± SD
		Sample	Addition Sample	Measurable Standard		
14	1	12,62	25,38	12,76	91,16	91,54 ± 0,486
	2	12,68	25,47	12,79	91,37	
	3	12,28	25,17	12,89	92,09	
18	1	12,62	29,84	17,23	95,70	102,76 ± 1,341
	2	12,68	29,98	17,30	96,12	
	3	12,28	29,96	17,68	98,23	
24	1	12,62	37,16	24,54	102,24	96,70 ± 0,953
	2	12,68	37,20	24,52	102,17	
	3	12,28	37,20	24,93	103,86	

Linearity

The results of determining the standard curve linear regression show the relationship between area size as a response and concentration, which can be seen in **table 3**

Table 3. Hydroquinone Linearity Test Results with Reversed Phase HPLC

Replication 1		Replication 2		Replication 3	
Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)	Area
12	509,3	12	486,9	12	504,4
14	611,1	14	609,5	14	588,3
18	781,9	18	782,7	18	773,8
24	1078,3	24	1068,1	24	1007
32	1383,2	32	1391	32	1365,3
A = -6,543		A = -29,845		A = -8,2248	
B = 43,965		B = 44874		B = 42,799	
r = 0,9986		r = 0,9987		r = 0,9996	

Precision

The results of precision testing can be seen in table 4 with the average results from 6 replications with a concentration of 18 ppm of RSD = 0.801%

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Table 4. Precision Test Results for Hydroquinone with Reversed Phase HPLC

Replication	Retention Time	Area
1	1,59	781,900
2	1,58	782,700
3	1,59	773,800
4	1,59	791,600
5	1,59	789,000
6	1,59	781,600
Average	1,586833	783,433
SD	0,003545	6,273
%RSD	0,223398	0,801

LOD (Limit Of Detection) and LOQ (Limit Of Quantitative)

The results of LOD and LOQ testing can be seen in table 5 LOD obtained 0.7 ppm and LOQ obtained 33.45 ppm.

Table 5. LOD and LOQ Test Results for Hydroquinone with Reversed Phase HPLC

No	Concentration (ppm)	Area(Y)	Yi=bx+a	$(Yi-Y)^2$
1	12	504,4	505,36	0,93
2	14	588,3	590,96	7,08
3	18	773,8	762,16	135,55
4	24	1007	1018,95	142,83
5	32	1365,3	1361,34	15,66
		Total		302,05
		LOD		0,7
		LOQ		33,45

System Suitability Test

The suitability results can be seen in **table 6** using 5 concentrations.

Table 6. Suitability Test Results of the Hydroquinone System with Reversed Phase HPLC

Replication	AUC	Retention Time	TF	N	HETP (cm)
1	781,561	1,588	1,21	4107	0,00365
2	781,870	1,589	1,25	4087	0,00367
3	773,797	1,588	1,22	4081	0,00368
4	791,669	1,588	1,22	4120	0,00364
5	788,999	1,587	1,24	4161	0,00360
X	783,579	1,588	1,228	4111	0,00365
RSD(%)	0,897	0,04452813	1,338084424	0,77615407	0,00772

Determination of Hydroquinone

The results of the hydroquinone content test on 8 whitening toners were replicated 3 times using HPLC with a retention time of 1.5 minutes. The levels obtained can be seen in **table 7**

Table 7. Results of Determination of Hydroquinone Levels

Sample	Replication	AUC	% Hydroquinone Levels (b/v)	Average (%) ± SD
1	-	-	-	-
2	1	15661,40	0,36612	0,36499 ± 0,00108
	2	15569,89	0,36398	
	3	15607,22	0,36486	
3	-	-	-	-
4	1	15154,85	0,35429	0,35248 ± 0,00197
	2	15090,55	0,35278	
	3	14987,85	0,35038	
5	-	-	-	-
6	1	531,78	0,01262	0,01253 ± 0,00022
	2	534,54	0,01268	

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	3	517,22	0,01228	
7	1	15383,77	0,35963	0,35271 ± 0,00603
	2	14966,48	0,34988	
	3	14912,60	0,34863	
8	1	144,36	0,00357	0,00371 ± 0,00018
	2	159,20	0,00391	
	3	147,82	0,00365	
9	1	268,88	0,00647	0,00516 ± 0,00142
	2	147,82	0,00365	
	3	221,22	0,00536	
10	-	-	-	-
11	-	-	-	-
12	1	192,96	0,00470	0,00272 ± 0,00172
	2	64,71	0,00170	
	3	66,45	0,00174	
13	1	57,139	0,00153	0,00152 ± 0,000027
	2	57,425	0,00153	
	3	55,294	0,00148	
14	-	-	-	-
15	-	-	-	-

In this study, 15 samples of the whitening toner used were coded T1-T15. These samples were obtained from the online marketplace X based on the criteria in this study. This study uses a reversed-phase because the mobile phase is relatively polar, and the stationary phase is nonpolar. It is known that the ε of hydroquinone is 3.104, so it can be analyzed using HPLC, which requires ε > 1,000. The value of A1% 1cm of hydroquinone at a wavelength of 295 nm is known to be 282 (Moffat, 2011). Hydroquinone can be analyzed at trace levels using HPLC with a UV-Vis detector. The hydroquinone colour reaction test using Benedict's reagent will undergo an oxidation-reduction reaction. If it is positive for hydroquinone, a red brick will be formed because hydroquinone is in the phenol group. From the study results, it was found that 8 samples were positive for hydroquinone due to changes. The results of this colour test can be confirmed by carrying out quantitative analysis using HPLC to determine the level of hydroquinone contained in the sample. Based on the results of the research that has been done, the maximum wavelength is 295 nm. This result is to the maximum hydroquinone length requirement of 293 ± 2 nm (Pharmacopeia, 2020). This maximum wavelength will be used to measure the absorbance of the whitening toner sample.

The results obtained from the specificity test have a resolution value of 4.042. These results meet the requirements because they have Rs ≥ 1.5 (Pharmacopeia, 2020). Based on the % recovery value obtained at 3 concentrations, it was 91.54%-102.76%, so the results obtained were by the accuracy requirements at a concentration of 10 ppm with a range of 80-110%. The results of the linear regression equation relate the hydroquinone concentration to the area obtained from the HPLC injection. The requirement for the coefficient of determination is r2 ≥ 0.999 (ICH, 2006). Of the 3 replications chosen, the best r2 value is replication 3 because it has the highest r2 value than the others, namely r2= 0.9994 and the value of r = 0.9996 with the equation Y = 42.799x-8.2248, then you get the results the calibration curve is linear and can be used for analysis of hydroquinone levels. From this research, the RSD result was 0.801%, which shows that the method used has good precision because the RSD or CV value is <2% (Pharmacopeia, 2020).

From this test that has been carried out, results were found for a LOD value of 0.7 ppm, which means that the tool is unable to detect if it is smaller than 0.7 ppm and if it is more significant than 0.7 ppm, the tool can detect with good precision and accuracy values. The LOQ value obtained was 33.45 ppm, which means that the tool can carry out measurements at this concentration with careful analysis results. This test is an integral part of the chromatography used to verify that the resolution and reproducibility of the HPLC system are sufficient for the analysis to be carried out with short retention times, resolution (Rs) ≥ 1.5, tailing factor (TF) ≤ 2 and theoretical plates (N) ≥ 3500 and meets the RSD value ≤ 2% (Pharmacopeia, 2020). Based on the results obtained in Table 6, the T2 sample obtained an average of 0.36499%; sample T4 0.35248%; sample T6 0.01253%; sample T7 0.35271%; sample T8 0.00371%; sample T9 0.00516%; samples T12 0.00272% and samples T13 0.00272%, while samples T1, T3, T5, T10, T11, T14 and T15 did not contain hydroquinone as evidenced by the colour test using Benedict's reagent. Based on BPOM NO 23 of 2019,

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hydroquinone levels are not allowed for cosmetics, and the research showed that the whitening toner sample did not meet BPOM requirements because it contained more than 0% hydroquinone.

Conclusions

The result of the 15 samples studied, 8 samples were positive hydroquinone, so it can be concluded that these samples do not meet the requirements of BPOM No. 23 of 2019

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