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ASSESSMENT ON HYROQUINONE IN SELECTED COSMETIC CREAM AND TONER VIA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ULTRA-VIOLET VISIBLE DETECTOR SPECTROMETRY

(Pengukuran Kandungan Hydroquinone di dalam Krim Kosmetik dan Penyegar Muka yang Terpilih Menggunakan Kromatografi Cecair Tekanan Tinggi dan Spektrometri Detektor Ultra-Violet Sinar Tampak)

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Abstract

Hydroquinone (HQ) is a whitening component in many cosmetic products, but only allowable at a very low concentration. The abuse of HQ will damage the skin and the sensitive area on the face. Due to this, many countries including Malaysia prohibited the use of HQ. However, the increasing number of whitening products in the market makes HQ suspiciously available. Thus, in this study, two quantitative methods of determining the HQ concentration were used. They were namely High Performance Liquid Chromatography (HPLC) and Ultra-Violet Visible Detector Spectrometry (UVDS). In the HPLC analysis, three types of mobile phase were used to separate three types of whitening products. The best mobile phase ratio was 20:80 v/v (methanol: water) due to the sharp peak of HQ and acceptable retention time (5.3 min). From the UVDS analysis, the measured HQ was found comparable to the HPLC results. Both methods were able to detect HQ lower than the permissible concentration. The HQ was also found to be stable for analysis in the same day and different days.

Keywords: cosmetic, HPLC, hydroquinone, UVDS, whitening

Abstrak

Hidrokuinon (HQ) adalah komponen pemutih di dalam produk kosmetik tetapi hanya dibenarkan pada kepekatan yang sangat rendah. Penyalahgunaan HQ akan merosakkan kulit dan kawasan sensitif di bahagian muka. Sehubungan dengan ini, banyak negara termasuk Malaysia telah mengharamkan penggunaan HQ. Walaubagaimana pun, peningkatan jumlah produk pemutih di pasaran membuatkan kecurigaan kandungan HQ. Selaras, di dalam kajian ini, dua kaedah kuantitatif dalam mengesan kandungan HQ telah digunakan. Kaedah yang dimaksudkan adalah Kromatografi Cecair Tekanan Tinggi (HPLC) dan Spektrometri Detektor Ultra-Violet Sinar Tampak (UVDS). Di dalam analisis HPLC, tiga jenis fasa gerak telah digunakan untuk mengasingkan tiga jenis produk pemutih. Fasa gerak terbaik adalah 20: 80 v/v (metanol: air) disebabkan oleh puncak HQ yang tajam dan waktu retensi yang boleh diterima (5.3 min). Daripada analisis UVDS, pengiraan HQ adalah setanding dengan keputusan HPLC. Kedua-dua kaedah mampu untuk mengesan HQ pada kepekatan yang dibenarkan. Analisis HQ juga didapati stabil pada hari yang sama dan hari berbeza.

Kata kunci: kosmetik, HPLC, hidrokuinon, UVDS, pemutih

Introduction

Hydroquinone (HQ) is synonymous to 1,4-Benzenediol and also 1,4-Dihydroxybenzene with the chemical formula $C_6H_6O_2$. HQ has a molecular weight of 110.11 g/mol. HQ is a white solid crystal with a melting point between 74-174 ^{0}C and a boiling point at 285 °C at 0.9605 atm [1]. HQ is harmful if swallowed and it may cause an allergic skin reaction, serious eye damage, suspected of causing genetic defect and also cancer. HQ compound does not only affect the skin, but it is also very toxic to aquatic life [2]. HQ can affect a person's health such as leukoderma-encofette, exogenous ochronosis and in the long term can cause carcinogenesis [3]. It is advisable to avoid any the solution that contains HQ that can be released to the environment. The precaution steps while handling with HQ are to wear protective gloves and goggles for eye protection [2]. DNA damages and mutations also can happen due to the oxidation of HQ into *p*-benzoquinone in the bone marrow [4]. HQ is also known as the most abundant organic constituents of mainstream tobacco smoke [5]. HQ can also reduce the protective mechanism ability in the body which is the side effect can cause cancer [4]. HQ is not allowed by the European Directive [6]. According to a published report [4], HQ has been banned in cosmetics since January 1, 2005. In Malaysia, the Ministry of Health also banned three beauty products due to them containing scheduled poisons, tretinoin and hydroquinone [7].

In the cosmetic world, HQ is well known as the depigmenting agent [8]. HQ is widely used in skin-toning creams. This is because HQ is believed to be effective in reducing face tone by decolourizing and preventing the formation of melanin in the skin. HQ can prevent the formation of melanin because of the toxicological effect of HQ [6]. Melanin can occur at the skin surface because of several factors such as genetic factors and also environmental factors such as the effect of UV sun radiation [9]. HQ is effective if the percentage of concentration present in any sample is between 1.5-2.0%, but will give side effects such as irritation, redness and burning if the percent of HQ concentration is higher than 5.0% [8]. The maximum amount of hydroquinone that is allowed in a cosmetic is 20 µg/ml [10]. Even though the U.S. did not establish a reference dosage, the Environmental Protection Agency has calculated the Provisional references of hydroquinone which is 0.04 mg/kg/d [11].

There are a few techniques to determine HQ in cream such as using High-Performance Liquid Chromatography (HPLC), spectrophotometry, Thin Layer Chromatography (TLC), and also Micellar Electrokinetic Chromatography (MEKC) [6]. Some other methods like chromatography is not suitable in analysing HQ because of several factors such as, the volatility factor, the polarity of sample factor, and also the thermal instability of the latter factor [9]. HQ sample has a viscous emulsion characteristic, so it is hard to isolate the analyte using simple separation technique [12]. In conducting a simple chromatography of HQ, TLC procedures may be used and the ratio of R_f can be calculated. For a better qualitative and quantitative study the best method is by HPLC [12]. This technique is well-known for conducting the separation and analytical methods [13]. Thus, in this experiment, HQ will be analysed using the HPLC method.

Materials and Methods

Chemicals and Standard Solutions

All the solvents used were HPLC grade. Hydroquinone and methanol were respectively purchased from Sigma Aldrich and R&M. A range of standard mixture stock solutions containing 2ppm - 14ppm were prepared in Millipore nylon fibre filtered mobile phase. The linearity of calibration curve was then obtained according to Beer's law.

HPLC System and UVDS

The chromatographic analysis was performed using a Waters 1515 Isocratic Pump with Waters 2487 Dual λ absorbance. The separation was done using a C18 column (100 mm x 2.1 mm) with the flow rate of 1.0 ml/min as suggested [8]. In order to study the effect of different mobile phase ratios on the separation of HQ, three ratios of mobile phase (water/methanol) solution had been used: 90 ml of water + 10 ml of methanol (90:10), 80 ml of water + 20 ml of methanol (80:20), and 70 ml of water + 30 ml of methanol (70:30). The spectroscopic analysis was done to compare the results obtained from the chromatographic analysis and it was done by using UV-Vis spectrophotometer. Blank for this analysis was distilled water, instead of sulfuric acid. The wavelength was set to 302.0 nm and a 1.0 cm quartz cell was used [8]. All analysis was done at ambient temperature.

Sample Preparation

The cosmetic samples used were toner and cream from different brands and were purchased from local stores and night market in Pahang. Liquid sample (toner) was weighted 0.1 gram. 100 ml of mobile phase was added and solution was mixed thoroughly. The solution was then transferred into a 100 ml volumetric flask and topped up the solution using mobile phase. The solution was filtered for prior analysis using HPLC. The procedures were repeated three times. UV detection was set at 295 nm [14]. On the other hand, for solid sample (cream), the sample was treated further after the solution has mixed homogenously. Similarly, 0.1 gram sample was weighted and 100 ml of mobile phase was added and mixed thoroughly. Next, the solution was placed in a water bath at 60°C for 10 minutes. The solution was again homogenized and then was cooled in an ice bath until the fats and wax appeared. The fats and wax were filtered using filter paper. The filter solution was transferred into a 100ml volumetric flask and the solution was marked up with the mobile phase. The sample solution was then analyzed using HPLC with the UV detection set at 295 nm [14]. All the procedures were repeated three times.

Results and Discussion

Mobile Phase Performance

Methanol was selected as the solvent. According to Lin et al. [12], methanol with suitable modifying solvents such as deionized water, was the most common solvents used for HPLC analysis during reversed-phase column. A report by Lin et al. [12] mentioned that decreased in the proportion of water, leads to decrease in the capacity factor. Hence, in this study, there were three types of mobile phase used. The compositions of mobile phase of water/methanol solution were: A = 70 ml of water with 30 ml of methanol (70:30), B = 80 ml of water with 20 ml of methanol (80:20) and C = 90 ml of water with 10 ml of methanol (90:10). Retention times for A were the lowest and A had the broadest peaks, followed by B. According to Nova'kova' et al. [15], once the fractions of water exceed 25% of the solvents used, the signal would be broad.

From mobile phase B, the peak was sharper and less broad compared to previous chromatogram. The value of retention time for HQ detected for this series of mobile phase was higher than previous retention time of composition of mobile phase A. The retention time would increase as the percentage of non-polar mobile phase decrease [16]. This happened when using a reversed phase chromatography. This situation can be seen from chromatogram at figure 4.3. The retention time for C was the highest compare to B and also A. Mobile phase with more water have more polarity characteristic. Hence, the value of retention time for HQ to be completely separated was the slowest.

Intraday and Inter-day Assessment of Mobile Phases

Intraday precision was obtained in the same day while for the inter-day precision was determined for three consecutive days. From previous study, concentration range obtained in cosmetic products using HPLC was 6 to 30 ppm [8]. They also stated that the precision of method was determined by the repeatability of the intra-day results. Hence, each of the samples was triplicated to get the precision of method. Intraday result of mobile phase A showed that, the average concentration obtained for B toner sample was 6.756 ppm. HQ concentration present in sample M toner was 4.766 ppm. While for the last cosmetic sample, which is M cream, the average concentration value obtained from three data recorded was 4.505 ppm. The retention time of this composition of mobile phase has the lowest value compare to other composition of mobile phase. While intraday result of mobile phase B, the average concentration was calculated. The average concentrations obtained for B toner and M toner samples were 8.333 and 3.898ppm respectively. While for the last cosmetic sample which is M cream was 4.570 ppm. For intraday result of mobile phase C, the average concentration obtained for B toner sample was 6.125 ppm. The HQ compound was detected at the early minute of 7. HQ concentration present in sample M toner was 4.435 ppm. While for the last cosmetic sample, which is M cream, the average concentration value obtained from three data recorded was 3.067 ppm. The summary of the results is shown in Figure 1.

The inter-day experiment was conducted in three consecutive days. The precision of the proposed method was determined by expressing the relative standard deviation (RSD) of each sample. The results of the respective RSD are displayed in Table 1. In previous research [10], the HQ concentration contained in their cosmetic sample tested was 24.84 ppm with the SD value 0.69 and percent of RSD was 0.29%, which is very small compared to this study.

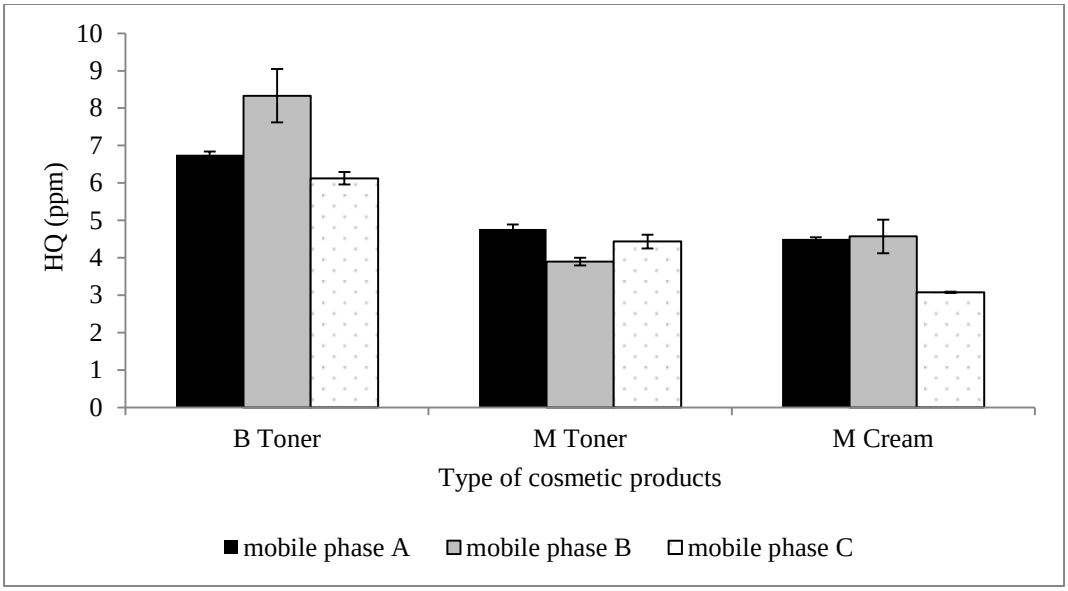

Figure 1. Hydroquinone concentration with three HPLC mobile phases for intraday analysis

Table 1. Value of RSD for samples

Sample Name	RSD (%)
B toner	9.66
M toner	3.90
M cream	2.13

Stability of HQ in Samples

The stability of the HQ compound in samples was determined by keeping the sample for one day and running the analysis of sample the next day. The stability test was conducted to access stability of HQ. The stability of B toner sample in mobile phase A showed that, the different value of concentrations between the intraday result and also the stability result was about 2.079%. While for sample M toner the average value was recorded and the value was 5.026 ppm. The percent different with the intraday result was 2.655%. While for sample M cream, the average value was recorded and the value was 4.717 ppm. The percent different with the intraday result was 2.298%. The stability of the HQ in B toner sample in mobile phase B showed that, the different value of concentrations between the intra-day result and also the stability result was about 3.042%. While for M toner sample in mobile phase B, the different value of concentrations between the intraday result and also stability result was about 0.671% and the result for M cream showed that, the different value of concentrations between the intraday result and also the stability result was about 5.950%. This showed that the HQ is a stable compound because the value of concentration recorded for the sample product was slightly the same.

LOD and LOQ

LOD's was calculated based on the standard deviation of the response or known as SD. The slope of the calibration curve (S) can represent the LOD according to the formula equation (1):

$$LOD = 3.3(SD/S) \quad (1)$$

Based on the standard deviation of y-intercepts of regression lines, the standard deviation of the response can be determined. The standard deviation of the response (SD) and the slope of the calibration curve (S) can be calculated based on the formula equation (2):

$$LOQ = 10(SD/S) \quad (2)$$

Again, based on the standard deviation of y-intercepts of regression lines from the equation, the standard deviation of the response can be determined. All of the values of SD and slope can be obtained from the linear function which is from the standard calibration graft. The standard deviation used for LOD and LOQ calculation came from the SD of equation. The quantitation and also detection limits were estimated for HQ compound. The values were calculated and result was obtained. The values obtained which indicated good sensitivity of the proposed HPLC method. A good linearity when the value of R^2 obtained was 0.9999 [3]. The value of LOD and LOQ obtained by a proposed method was 0.08 µg/ml and 0.26 µg/ml, respectively [8]. In the present study, the values of LOD and LOQ for each mobile phase were show in Table 2.

Table 2. Limit of Detection and Quantification of for each mobile phase.

Mobile Phase	**Equation Y = SX + C**	**r^2 Mean (± S.D.)**	**LOD (µg/ml)**	**LOQ (µg/ml)**
A	Y = 2.20e+004X +7.99e+003	0.999	7.77×10^{-2}	2.35×10^{-1}
B	Y = 2.42e+004X +8.92e+003	0.994	7.08×10^{-2}	2.14×10^{-1}
C	Y = 2.25e+004X +8.46e+002	0.997	7.60×10^{-2}	2.31×10^{-1}

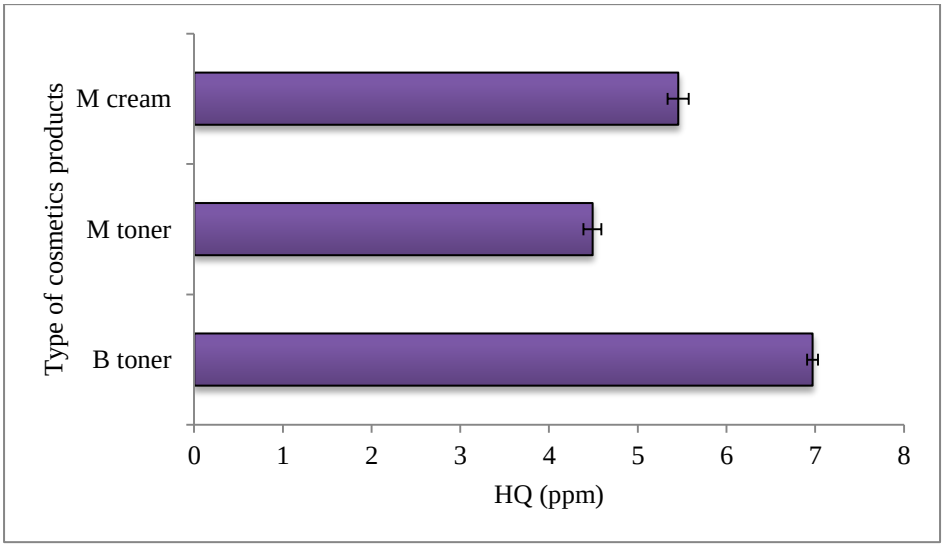

Figure 2. Hydroquinone concentration in samples by UVDS analysis

UVDS Analysis

It was mentioned that UVDS methods allow a low cost quantitative determination of the HQ [8]. From previous study, the concentration range of the HQ detected was 10 to 26 ppm with the regression equation y= 0.01756x + 0.00142 and the correlation coefficient was 0.9999 [8]. In this study, the analysis of the HQ concentration by UVDS is shown in Figure 2. The regression equation obtained was y= 0.00924x + 0.0491 with the correlation coefficient 0.99802. From the previous study, they used sulfuric acid as the blank solution, and the percent of RSD value obtained was 0.97% [8]. By using distilled water as the blank solution, the percent of RSD value obtained for M toner was 1.78%, while for B toner was 2.26% and for M cream was 1.72%.

Conclusion

From the empirical data, the HQ can be found in all samples relative to the labeled value. In short, compared to the packaging information of the respective products, the B toner contained higher HQ concentration, the M toner had slightly the same concentration and in M cream HQ was found present although it was not labeled in the information. Besides that, a simple method by the UVDS analysis can be an alternative to the HPLC methods where running the sample without sulfuric acid is possible. The effect of the mobile phase ratio on the HQ separation process was also determined. The most efficient mobile phase in this study was methanol: water in composition of 20:80. This is because of the respective mobile phase can give the sharpest peak in detection of the HQ. The stability of the HQ compound also proved that the HQ compound is a stable compound. Beyond the limitation of time and facilities, it is vital to investigate more cosmetic products so that people would be aware of the presence of HQ and its consequences towards skin health.

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References

1. The National Institute for Occupational Safety & Health (1978). Criteria for a Recommended Standard: Occupational Exposure to Hydroquinone. Retrieved from http://www.cdc.gov/niosh/docs/1970/78-155.html.
2. Sigma-Aldrich, (2014**).** Hydroquinone ReagentPlus®, ≥99%. Retrieved from http://www.sigmaaldrich.com/catalog/product/sial/h9003?lang=en&region=MY
3. Thongchai, W., Liawruangrath, B. & Liawruangrath. S. (2007). High-performance Liquid Chromatographic Determination of Arbutin in Skin-whitening Creams and Medicinal Plants Extracts. *J. Cosmet. Sci., 58*: 35-44.
4. Westerhof, W. and Kooyers, T.J. (2005). Hydroquinone and its Analogues in Dermatology – A Potential Health Risk. *Journal of Cosmetic Derm*atology 4(2): 55–59.
5. Zhao, L., Baoqiang, L., Yuan, H., Zhaou, Z. and Xiao, D. (2007). A Sensitive Chemiluminescence Method for Determination of Hydroquinone and Catechol. *Sensor* 7: 578-588.
6. Desiderio, C., Osscini, L. and Fanali, S. (2000). Analysis of Hydroquinone and Some of its Ethers by Using Capillary Electro chromatography. *J Chromatography A, 887*: 489-496.
7. Health Ministry Bands Three Products. (2010). Retrieved from http://www.theborneopost.com/2010/05/18/health-ministry-bans-three-beauty-products/
8. Garcia, P.L., Santoro, M.I.R.M., Kedor-Hackman, E.R.M. and Singh, A.K. (2005). Development and Validation of a HPLC and UV Derivative Spectrophotometric Methods for Determination of Hydroquinone in Gel and Cream Preparation. *Journal of Pharmaceutical Biomedical Analysis* 39: *764-768.*
9. Gao, W. and Legido-Quigley, C. (2011). Fast and Sensitive High Performance Liquid Chromatography Analysis of Cosmetic Creams for Hydroquinone, Phenol and Six Preservatives. *Journal Chromatography A 1218*: 4307-4311.
10. Wang, A.C., Cheng, S.H., Sheu, C. & Kwan, C.C. (2011). Simultaneous Determination of Five Whitening Agents by Ion-Pair Reversed-Phase High Performance Liquid Chromatography. *International Journal of Applied Science and Engineering* 9 (4): 287-299.
11. Odumosu, P.O. and Ekwe, T.O. (2010). Identification and Spectrophotometric Determination of Hydroquinone levels in Some Cosmetic Creams. *African J Pharmacy and Pharmacology, 4(5)*: 231-234.

12. Lin, W.C., Lin, S.T. and Shu, S.L. (2000). Comparison of Analyses of Surfactants in Cosmetics Using High-Performance Liquid Chromatography and High-Performance Capillary Electrophoresis. *Journal of Surfactants Detergents* 3(1): 67-72.
13. Bielicka-Daszkiewicz, K., Hadzicka, M. and Voelkel. A. (2012). Optimizing of SPE/GC/HPLC Analytical Procedure for Determination of Phenol, Quinones, and Carboxylic Acids in Water Samples. *ISRN Chromatography* 2012: 1-7.
14. Parveen, Z., Siddique, S., Ali. Z. and Zaheer. M. (2012). Qualitative and Quantitative Estimation of Hydroquinone in Skin Whitening Cosmetics. *Journal of Cosmetic Dermatological Science and Applications* 2: 224-228.
15. Nova'kova', L., Solich, P. and Solichova, D., (2008). HPLC Methods for Simultaneous Determination of Ascorbic and Dehydroascorbic Acids. *Trends in Analytical Chemistry* 27: 942-958.
16. Bagheri, H., Mohammadi, A. and Salemi, A., (2004). On-line trace enrichment of Phenolic Compounds from Water using a Pyrrole-Based Polymer as The Solid-Phase Extraction Sorbent Coupled with High-Performance Liquid Chromatography. *Analytica Chimca Acta* 513(2): 445-449.