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Comparison of *Eco - Scale* Between UV - Vis Spectrophotometry and Thin Layer Chromatography - Densitometry Methods on The Determinination of Retinoic Acid in Facial Cream Preparations

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Abstract: Retinoic acid, a compound derived from vitamin A and its active metabolites, is often found in cream preparations and can be used without a doctor's prescription. This research aims to analyze the retinoic acid content in facial cream preparations and determine the environmental friendliness of the analytical method used. The materials used in this research were PBFI retinoic acid, methanol, TLC plates, n-hexane, acetone, and three facial cream samples. UV - Vis Spectrophotometry and Thin Layer Chromatography - Densitometry are methods used for assay analysis and Eco - Scale is a method for determining greenness. The research found that three cream samples tested positive for retinoic acid, which has been banned by BPOM since 1999. The UV-Vis Spectrophotometry method measured retinoic acid content of 0.103%, 0.266% and 0.256%, with a total score of 24 points. The Thin Layer Chromatography - Densitometry method measured retinoic acid content of 0.0213%, 0.0314%, and 0.027%, with a score of 50 points. It can be concluded that the UV - Vis Spectrophotometry method is more environmentally friendly compared to the Thin Layer Chromatography - Densitometry method based on Eco - Scale analysis.

Keywords: *Eco – Scale,* UV – Vis Spectrophotometry, Thin Layer Chromatography – Densitometry, Retinoic Acid, Facial Cream

INTRODUCTION

Retinoic acid, derived from vitamin A and its active metabolite all-trans retinoic acid (ATRA) is a potent antiaging agent used to treat skin conditions like wrinkles and hyperpigmentation (Szymański Ł , 2020). Retinoic acid can only be used with a doctor's prescription, however in practice retinoic acid is often purchased without a prescription and is sold freely. Indonesian regulations prohibit the use of retinoic acid or tretinoin in cosmetics due to concerns about dry skin, irritation, and birth defects. Retinoic acid is often found in facial whitening cream preparations, labeled as tretinoin. Consumers pay without considering long-term safety, and online trading has led to rogue manufacturers distributing these products without a distribution license.

The study focuses on the analysis of retinoic acid using UV-Vis Spectrophotometry, which has been widely used in research. The method has several advantages, including a well-structured procedure and good data quality. An alternative method Thin Layer Chromatography was also used, which is valid according to the BPOM guideline (Ditjen POM, 2011). However, the study does not consider the concept of green chemistry or the greenness. The greenness of the method should be evaluated using methods such as the National Environmental Methods Index (NEMI), Assessment of Green Profile (AGP), Green Analytical Procedure Index (GAPI), and Eco-scale (El-Naem & Saleh, 2020).

NEMI provides data to identify a method by providing a piktogram as an indicator of greenness. AGP is a semi-kuantitatif analysis that provides more parameters than NEMI, while GAPI ensures more parameters from AGP using a five-point scale. Eco-scale is a method that can measure the greenness of a sample using a 1-100 scale, focusing on parameters such as reagent, house, energy, and language. The study uses Eco-scale because it offers a more efficient method compared to other methods (Yabré, 2020).

METHODS

Material and Methods

This research was conducted at the organic chemistry laboratory Sekolah Tinggi Ilmu Farmasi (STIFAR) Semarang and chemistry laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang.

UV – Vis Spectrophotometry

The UV-Vis spectrophotometry analysis involved creating a 1000 ppm retinoic acid standard solution, a 100 ppm retinoic acid standard, and a 10 ppm retinoic acid solution. The absorbance values were measured in the 200-400 nm wavelength range, and a calibration curve was created. The correlation coefficient (r) was obtained from the curve, which was used to determine the sample concentration. A 3 grams facial cream sample was weighed, mixed with methanol, and filtered. The results were cooled, filtered, and pipetted. The absorbance was measured at the maximum wavelength of 330 nm.

Thin Layer Chromatography - Densitometry

Silika Gel 60F254 is used for a chromatographic analysis, with a 20 cm x 20 cm plate and 1 cm thickness of apex and base. The chromatographic phase B is created from n-heksan and aseton (6:4) v/v with a 50 mL volume and is used for the chromatographic process. The TLC plate is prepared by making a spotting boundary and an elution boundary approximately 1 cm from the edge of the TLC plate. Give a marking limit on the TLC plate for standards and samples with a distance of 1 cm. The spotting process uses a linomat 5 tool, rinse the linomat needle as much as 1 µL using methanol before dotting a standard solution of retinoic acid with a concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm as much as 2 µL and 5 µL of sample. Densitometri analysis is performed by placing the TLC plate in a densitometer, and the densitogram is plotted on a liner regression plot. The results can be used to determine the retinol content in the sample.

Eco – Scale

Eco-Scale analysis is a method used to determine the safety of a product by assigning penalty points to each parameter[4]. The number of pictograms for each reagent is determined using the danger symbol from the MSDS, multiplied by the hazard penalty points. The electrical energy parameters for each sample are determined using the UV-Vis spectrophotometry method and the TLC – Densitometry method[5]. The total score of penalty points determines the Eco-Scale value.

RESULT AND DISCUSSION

This research aimed to determine the retinoic acid content in free-to-sell face creams without BPOM certification, as the use of retinoic acid in beauty creams has been banned since 1998 due to miscarriage cases. The study used UV-

Vis Spectrophotometry and Thin Layer Chromatography-Densitometry methods, considering green chemistry and Eco-Scale analysis. From the measurement results it was found that the three samples analyzed were positive for containing a high percentage of retinoic acid. The analysis results of each method can be seen in the table 1.

Table 1. Results of Quantitative Analysis UV – Vis Spectrophotometry

Sample	Replication	Absorbance	Concetration (ppm)	Percentage (%)	Average Percentage (%)
X	1	0,406	6,8910	0,11	
	2	0,385	6,5276	0,10	0,103
	3	0,373	6,3200	0,10	
Y	1	0,51	8,6903	0,27	
	2	0,499	8,5	0,27	0,266
	3	0,479	8,1539	0,26	
Z	1	0,473	8,0501	0,26	
	2	0,48	8,1712	0,26	0,256
	3	0,455	7,7387	0,25	

UV-Vis spectrophotometry is a method used to analyze substances in samples using visible light interactions. Retinoic acid absorbs ultraviolet radiation due to its chromophore and auxochrome groups, causing it to have color. A blank solution was used as a control to ensure accurate measurements. Retinoic acid compound value is obtained at maximum wavelength of 330 nm have an absorbance value of 0.459. A standard curve was created an even scale from a concentration of 1 - 10 ppm with the absorbance value subtracted from the methanol blank. The linear equation y = 0.0578x + 0.0077 was used to determine the concentration of each cream sample suspected to contain retinoic acid.

Retinoic acid is soluble in organic solvents like ethanol, methanol, and chloroform, but insoluble in water or glycerol (Friese *et al*., 2020). Methanol is used as a solvent due to its higher extraction ability and lower boiling point. The container was coated with aluminum foil to protect the sample from direct light exposure (Shields *et al*., 2018). The retinoic acid-methanol phase was taken and filtered using Whatman No. filter paper to separate the sample solution from other components. The retinoic acid-methanol phase was diluted with methanol to obtain the sample concentration results.

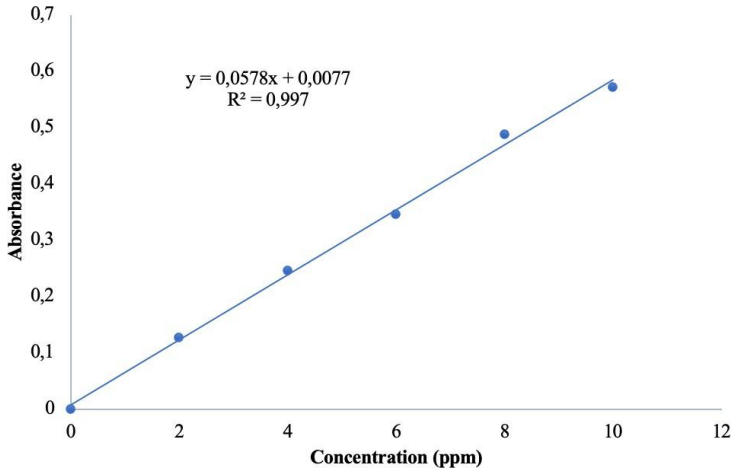

Figure 1. Retinoic Acid Standard Curve UV – Vis Spectrophotometry

Analysis of Retinoic Acid with TLC – Densitometry. The study analyzed the presence of retinoic acid in facial cream preparations using both qualitative and quantitative methods. The qualitative analysis involved determining the Rf value of visible spots on the TLC plate under UV light at a wavelength of 254 nm, while the quantitative analysis determined the concentration of the analyte in the sample using identification of the area on the chromatogram with densitometer.

Table 2. Results of Qualitative Analysis of TLC Retinoic Acid Cream Samples

Sample	Replication	Rf Value	UV Lamp $_{245}$	Result
X	1	0,77	Dark spots	+
	2	0,77	Dark spots	+
	3	0,77	Dark spots	+
Y	1	0,76	Dark spots	+
	2	0,77	Dark spots	+
	3	0,77	Dark spots	+
Z	1	0,78	Dark spots	+
	2	0,79	Dark spots	+
	3	0,80	Dark spots	+

Notes :
(+) = showed positive presence of retinoic acid compounds

The mobile phase was determined using two different motion phases: mobile phase A (n-hexane – 0.33% glacial acetic acid in ethanol p.a) and mobile phase B (n-hexane – acetone (6:4) v/v). Mobile phase A did not produce visible spots on the TLC plate, while mobile phase B produced visible spots. Mobile phase B was found to be better than mobile phase A due to its higher solubility and volatility.

The stationary phase used was silica gel 60 GF 254, which has polar properties. Samples with polar properties have greater binding to the polar mobile phase, allowing them to dissolve more slowly and move up the TLC plate more slowly (Skoog *et al*., 2013). Nonpolar samples move up the TLC plate more quickly due to reduced interaction with the polar stationary phase. After obtaining the best mobile phase, the process continued by developing mobile phase B in the chamber to ensure homogeneous conditions in the chamber, allowing the elution process to run smoothly and avoid a tail in the stain.

The study focuses on the detection of retinoic acid in facial cream samples using a fluorescent TLC plate. The standard solution did not show any spots under 254 nm UV light, but three cream samples formed dark blue stains, indicating the presence of retinoic acid compounds. The Rf value of the sample was compared to the standard Rf value of retinoic acid, and if the two Rf values are the same, the compounds are considered similar. The silica gel in the TLC plate fluorescent and re-emits it as visible light making the entire surface shine brightly under UV light. Retinoic acid can be detected under UV light due to its conjugated double bond system which allows it to absorb light energy in the ultraviolet range and emit light at longer wavelengths. After the sample is spotted, the retinoic acid compound in the sample absorbs UV light, extinguishing the fluorescence on the silica gel plate causing dark spots where the sample is spotted (Hadriyati, 2021).

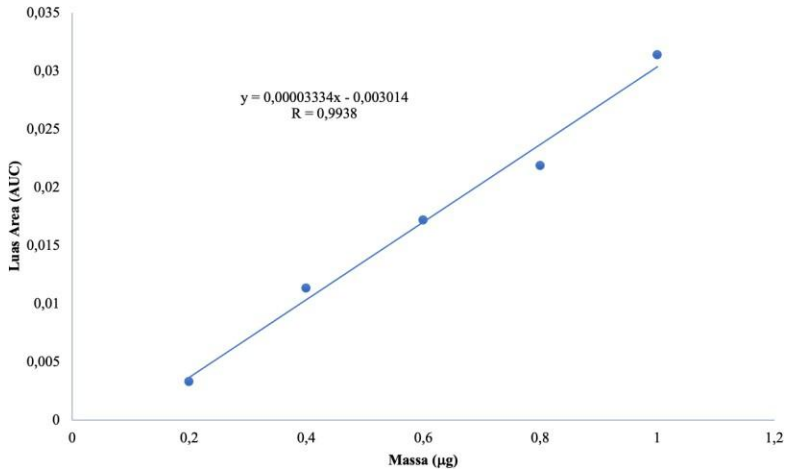

Figure 2. Retinoic Acid Standar Curve TLC – Densitometry

Table 3. Result of Quantitative Densitometry

Sample	Replication	AUC	Mass (μg)	Percentage (mg/L)	Percentage (%)	Average Percentage (%)
X	1	0,00947	374,51	74,90	0,0267	
	2	0,00580	264,36	52,87	0,0179	0,0213
	3	0,00713	304,25	60,85	0,0194	
Y	1	0,01659	588,00	117,60	0,0391	
	2	0,01058	407,73	81,54	0,0273	0,0314
	3	0,01181	444,6	88,92	0,0279	
Z	1	0,01162	438,93	87,78	0,0291	
	2	0,00962	378,94	75,78	0,0240	0,027
	3	0,01113	424,23	84,84	0,0279	

Table 4. Result of Eco – Scale Analysis

Parameter	Poin penalti (PP)	
	Spektrofotometri UV–Vis	KLT–Densitometri
Reagen		
Metanol	18	18
n – heksan	-	16
Aseton	-	8
Instrumen		
Energi	0	0
Resiko pekerja	0	0
Limbah	6	8
Total PP	24	50
Skor *Eco-Scale*	100 – 24 = 76	100 – 50 = 50

Quantitative scanning using a densitometer instrument was conducted to measure the chromatogram density of the sample separately and compare it with the standard area on the TLC plate. The highest absorption of the retinoic acid standard was at a wavelength of 337 nm. The Area Under Curve (AUC) was then converted into a standard curve equation, showing a linear relationship between the standard concentration of retinoic acid and the AUC. The regression equation for retinoic acid levels in cream samples was calculated using the accepted r value parameter of ≥ 0.99.

The analysis of retinoic acid levels in cream using UV-Vis Spectrophotometry and TLC-Densitometry methods revealed significant differences in concentration results. These differences were attributed to several factors, including instrument errors, method errors, and researcher errors. Instrument errors occurred when the densitogram was not completely shaded, causing the analysis to not be optimal. Method errors were due to differences in sensitivity between UV-Vis Spectrophotometry and TLC-Densitometry. Potential impurities in the sample also interfered with the analysis process, requiring repeated filtration.

Research by Maulana & Ghozali (2020) found significant differences in caffeine levels between the two methods. Cahyono *et al.* (2020) found similar trends in DPPH radical scavenging activity quantification. Therefore, it is unclear which method is more valid for analyzing caffeine in fat burning supplement samples. Additional tests, precision and accuracy tests, and sample probability based on standard deviation (SD) value are necessary to ensure the validity of the analysis method.

Despite the differences in levels, both UV-Vis Spectrophotometry and TLC-Densitometry methods show the presence of retinoic acid content in cream samples. Retinoic acid is prohibited for cosmetic use due to its potential teratogenicity.

Analysis of Eco – Scale, this research uses two analytical methods that is UV-Vis Spectrophotometry and TLC-Densitometry to determine retinoic acid levels in cream preparations. The environmental impact of these methods is measured using eco-scale (Yabré *et al*., 2020). The reagent parameter assessment is divided into two parameters: the number of reagents used and the danger of the compound. Methanol was used as the standard solvent in both methods, with a total penalty point score of 18 points.

The instrument assessment is divided into three parameters: the electrical energy required by the instrument/tool to analyze the sample, which received the same penalty points as the UV-Vis Spectrophotometry method. The worker risk parameter was categorized into two: analysis of the hermetization process and emissions of steam and gas into the air. Both methods received the same penalty points for the worker risk parameter.

The waste produced by the tool/instrument was categorized into eight categories with two classifications. The UV-Vis Spectrophotometry method produced 3 mL of waste, with a penalty point of 3 and the absence of treatment on the waste. The TLC-Densitometry method produced 50 mL of waste, with a penalty point of 3 for the absence of waste treatment.

The UV - Vis Spectrophotometry method has a total penalty score of 24 points, indicating its excellent green analysis for retinoic acid levels. The KLT - Densitometry method has a score of 50 points, indicating its acceptable green analysis. The UV - Vis Spectrophotometry method is more environmentally friendly, as it uses only one reagent to create the mobile phase in TLC. This aligns with the Indonesian Pharmacopoeia Ed VI's recommendation for retinoic acid analysis using the ultraviolet absorption spectrum method. The TLC - Densitometry method has a higher reagent score as it uses more reagents to create the mobile phase. The more stages in an analytical procedure, the less environmentally friendly the procedure is because increases the use of reagents and waste (Tobiszewski *et al*., 2017).

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the retinoic acid content in facial cream preparations was measured using the UV – Vis Spectrophotometry method from the three samples, namely sample x was 0.103%, sample y was 0.266%, and sample z was 0.256%. Meanwhile, what was measured using the Thin Layer Chromatography - Densitometry method from the three samples, namely sample x was 0.0213%, sample y was 0.0314%, and sample z was 0.027%. The results of the three samples were positive for containing retinoic acid which is not in accordance with BPOM regulations, where its use has been prohibited since 1998. The penalty point value for the UV – Vis Spectrophotometry method for chemical compound parameters is 18 points and for instrument parameters is 6 points. Meanwhile, the penalty point value for the Thin Layer Chromatography - Densitometry method for chemical compound parameters is 42 points and instrument parameters is 8 points. The UV – Vis Spectrophotometry method is a more environmentally friendly method compared to the Thin Layer Chromatography – Densitometry method based on Eco – Scale analysis.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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