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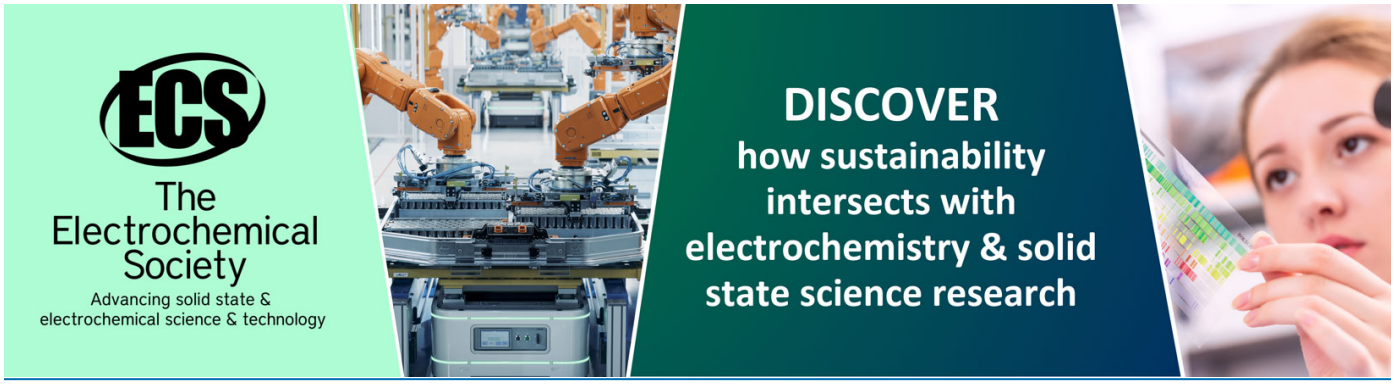

The Development of Analytical Method for the Determination of Azelaic Acid Content in Cosmetic Cream Products

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Abstract. Azelaic acid is one of the substances that has anti-acne and skin lightening effects which is often added to cosmetics. In the acne treatment, the azelaic acid is generally used with a concentration of 20% in cream formulation and 15% in gel. The use at concentrations below 10% is not recommended because it does not work effectively. While the use of above 10% is categorized as a medical treatment. In Indonesia, the Head of the National Agency of Drug and Food Control (BPOM) has issued Regulation No. 18 of 2015 on the Technical Requirements of Cosmetics Ingredients Annex V stating that the azelaic acid is banned in cosmetics. However, until this research began the BPOM has not had a valid method to identify it in cosmetics. Consequently, surveillance of such ingredient in products is hard to do. In this research, the fatty acid standard analysis method of AOAC International was modified and validated to be used in the laboratory. The method of analysis involves heating the cream preparations dissolved with methanol and then added BF_3-methanol catalyst, followed by extraction and analysis using GCMS. The validation of method shows that the calibration curve is linear with correlative value of 0.9997. The method is fairly sensitive with 0.02% detection limit, and fairly precision with relative standard deviation (RSD) of between 0.626-0.961% and fairly accurate which the recovery percentage is 99.85% at range 98.27-100.72%. In sum the results demonstrate that the method can be used as a routine analysis method for laboratory testing.

Key words: Azelaic acid, Dimethyl azelate, Derivatization, BF_3-Methanol, GCMS, Validation of analytical methods.

1. Introduction

Azelaic acid (1,7-heptane-dicarboxylic acid) with the molecular formula of $C_9H_{16}O_4$ is a saturated dicarboxylic acid. It is naturally present in yeasts Pityrosporum ovale, rice grains, milk and castor oil. Figure 1 shows the structure of azelaic acid. Azelaic acid is used for the local treatment of various types of cystic acne, including inflammation caused by acne, as an alternative to topical treatment with retinoic, benzoyl peroxide and erythromycin.

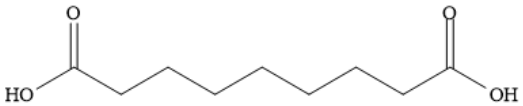


Figure 1. The structure of azelaic acid

Determination of dicarboxylic acid posesses a great challenge because of the absence of chromophores and fluorophore in this compound [1]. This compound also has a low volatility, so it needs derivatization before it is analyzed by gas chromatography [2].

This paper describes the development of a method to analyze azelaic acid in cosmetic products by means of derivatization through boron trifluoride (BF_3) in methanol esterification and the results were analyzed by gas chromatography mass spectrometry (GCMS). Derivatization methods with these reagents are easy and inexpensive to form esters which most often used to form methyl esters by reaction with an acid [3].

2. Experimental

2.1. Chemicals and samples

Azelaic acid, purity > 99% of Sigma Aldrich is used as standard. Methyl docosanoate, purity > 99% of Sigma Aldrich is used as an internal standard to minimize the possibility of deviation during the injection process into gas chromatography. 10% boron trifluoride in methanol (BF_3-methanol) solution from Sigma Aldrich is used as a degrading reagent for analysis with gas chromatography. LC grade methanol from Merck is used as a solvent to prepare standard solutions and test solutions. N-Hexane is used in extraction. Sodium chloride is used to produce saturated solutions which also used in the extraction. Sodium sulfate anhydrate pro-analysis degrees from Merck is used to pull water from the extract solutions. 90 mm diameter filter paper from Whatman is used to filter the solutions. Anti-acne cream sample which does not contain azelaic acid is self-made in the laboratory by following cosmetic cream-making procedures. The formulations contain compositions commonly present in cosmetic samples such as emulsifiers, refiners, moisturizers, pH regulators and solvents.

2.2. Test Method

2.1.1. Preparation of Standard Solution and Test Solution. The derivatization is carried out by following the fatty acid and oil derivatization methods listed in AOAC [4] with some adjustment of pipette 2 ml of the solution and transferred into the threaded cap erlenmeyer, 4 mL of BF_3-methanol 10% is added into the solution, afterward the solution is heated over the water bath at 60 ^{O}C for 10 minutes. The solution is cooled until the room temperature, then is moved into separating funnel. 10 mL of saturated NaCl is added into the solution and shaken for 15 seconds. 10 mL n-Hexane is added into the solution, then followed by extraction for 1 min. Next the n-Hexane layer is taken. Hereinafter the solution is strained through the filter paper that has been added sodium sulfate anhydrate. The filtrate is then taken as much as 1 mL. The filtrate is inserted into the vial auto sampler and 0.5 mL of internal standard solution is added, followed by homogenization and analysis with GCMS.

2.1.2. Instrument. Analysis GCMS is carried out on GCMS-QP 2010 plus system (Shimadzu) with AOAC 2000 autosampler. The GCMS experimental conditions for azelaic acid content as shown in Table 1.

Table 1. GCMS Experimental Conditions

Column	VF-wax MS (30 m, 0.25mm i.d., 0.25 µm film thickness)
Injector temperature	250 ^{O}C
Carrier gas	Helium
Carrier gas flow	1 mL/min
Split ratio	Splitless
Oven programme	150 ^{O}C
	20 ^{O}C/min 205 ^{O}C 2 min
	20 ^{O}C/min 250 ^{O}C 5 min
	Total run time: 14.75 min
MS Parameters:	
Ionization source	EI

Electron energy	70 Ev
Source temperature	180 ^{O}C
Interface temperature	250 ^{O}C
Fragment scan range	30 m/z – 220 m/z
Solvent cut time	4.01 minutes

2.1.3. Validation Method Parameters. The validation method is done by evaluating specificity, precision, accuracy, linearity, limit of detection and limit of quantitation [5].

3. Results and Discussion

3.1. The Study of BF_3-Methanol Volumes and Reaction Temperatures

The reaction between an azelaic acid (a carboxylic acid) with methanol (alcohol) requires BF_3 as an acid catalyst which produces dimethyl azelate (ester) is called an esterification reaction, as shown in Figure 2. Esterification involves heating the carboxylic acid with an acid catalyst in an alcohol solvent. The catalyst protonates an oxygen atom of the COOH group, making the acid much more reactive to nucleophiles. An alcohol molecule (CH_3OH) then combines with the protonated acid, to yield the ester product (R-COO-CH_3) with the lost of one water molecule. Esterification is a reversible reaction. To obtain a high yield of the ester, the equilibrium should be shifted towards the ester side. One technique for achieving it is by using one of the reagents excessively.

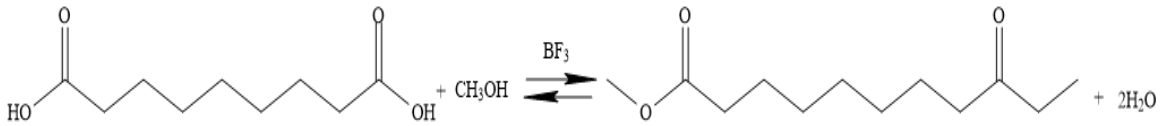
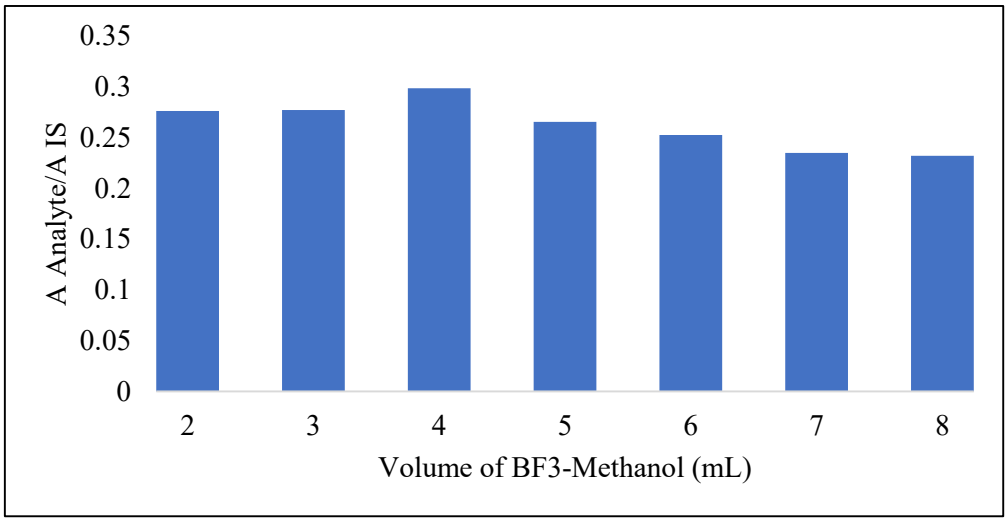
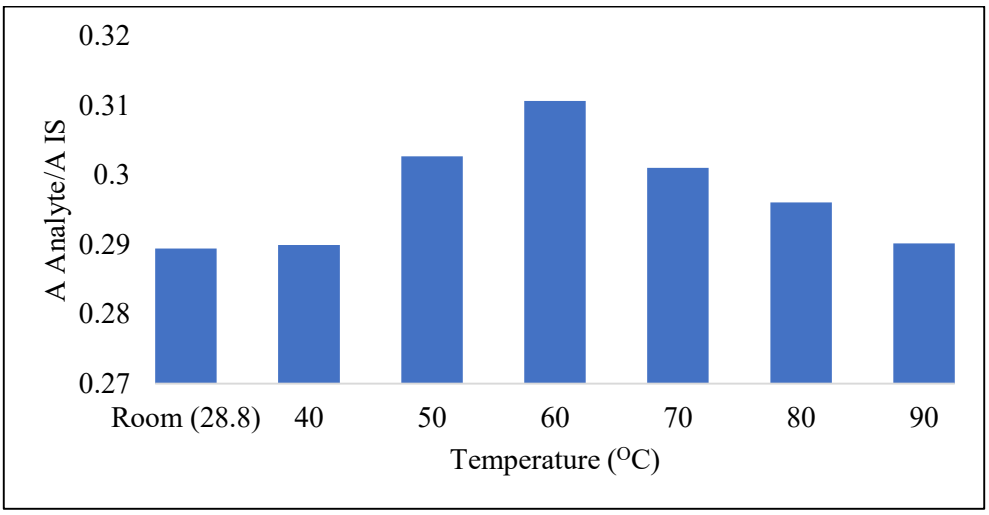


Figure 2. Esterification reaction of azelaic acid

From the equation of the reaction it is apparent that an appropriate amount of BF_3-methanol is required for the right shift reaction and the proper reaction temperature to accelerate the reaction time but not reduce the amount of the present. The result of the analysis is shown in Figure 3.



(a)



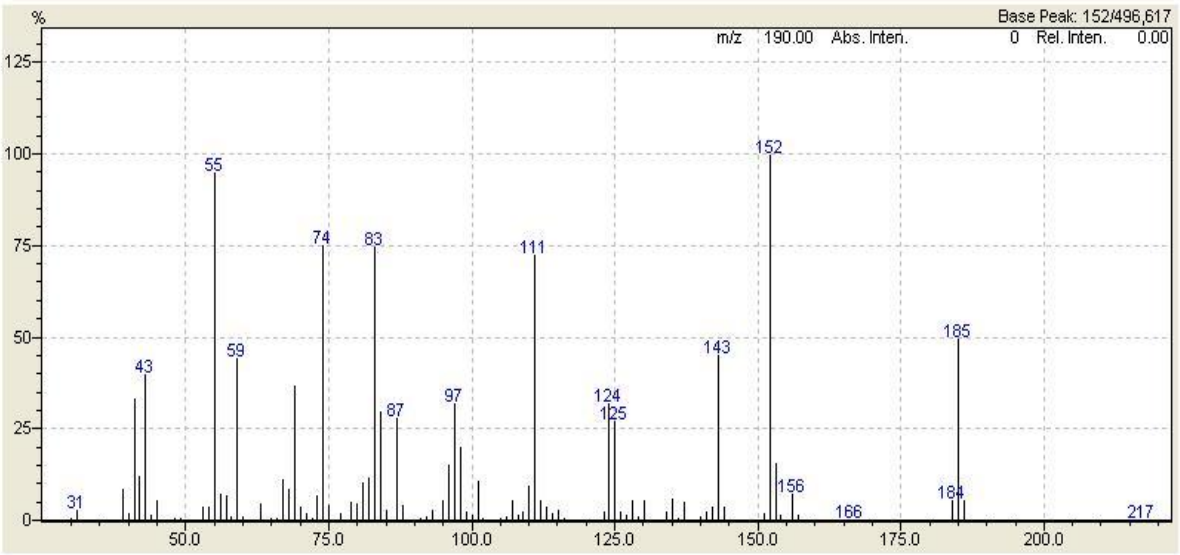
(b)

Figure 3. Effects of volume (a) and temperature (b) on yield of the derivatization results

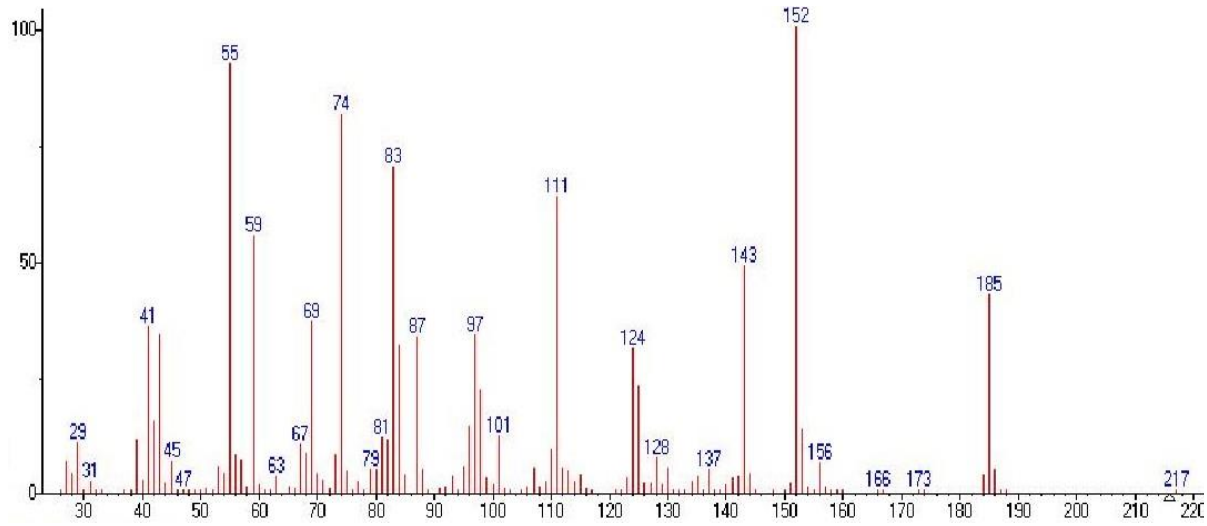
From Figure 3 it is clearly seen that 4 mL of BF_3-methanol and heating at 60 ^{O}C was the optimal condition for this derivatization method.

3.2. Mass Spectral Analysis

According to the analysis performed with GCMS the retention time of dimethyl azelate was in the range 6.0 to 6.3 respectively (Figure 5(b)). The analyte spectrum match to the reference spectrum of NIST. The mass spectrum and reference mass spectrum of the dimethyl azelate are shown in Figure 4. From the mass spectra profile is obtained that the parent peak is $[M+1]^+$ 217. The most abundant ions are in the high mass range, *m/z* 185 ($[M\text{-}CH_3O]^+$), *m/z* 152 ($[M\text{- 2 x }CH_3O]^+$), *m/z* 143 ($[M\text{-}CH_3OCOCH_2]^+$), *m/z* 124 ($[M\text{-}(CH_3OCO+CH_3O+2H]^+$), and *m/z* 111 ($[M\text{-}(CH_3OCOCH_2+CH_3O+H]^+$). Ions equivalent to *m/z* 156 ($[M\text{-}CH_3OCO+H]^+$) seem to be characteristic of shorten-chain dibasic esters [6].



(a)



(b)

Figure 4. (a) Mass spectrum of azelaic acid dimethyl ester and (b) Reference mass spectrum of azelaic dimethyl ester in NIST

3.3. Selectivity

Selectivity is the ability of a method to explicitly test the analyte in question by the presence of other or predicted components such as contamination, degradation, and other components that may be present in the sample matrix. The specificity of proposed method is justified by the chromatograms of placebo, azelaic acid with internal standard methyl docosanoate and spiked sample solution under same chromatographic conditions show in Figure 5. The placebo is an anti-acne cream sample without azelaic acid and internal standard methyl docosanoate while the spiked sample is spike of placebo with azelaic acid and internal standard methyl docosanoate. The placebo did not interfere in determination of Azelaic acid in commercial cream. The retention time for azelaic acid was about 6.118 minutes and for internal standard was about 12.718 minutes.

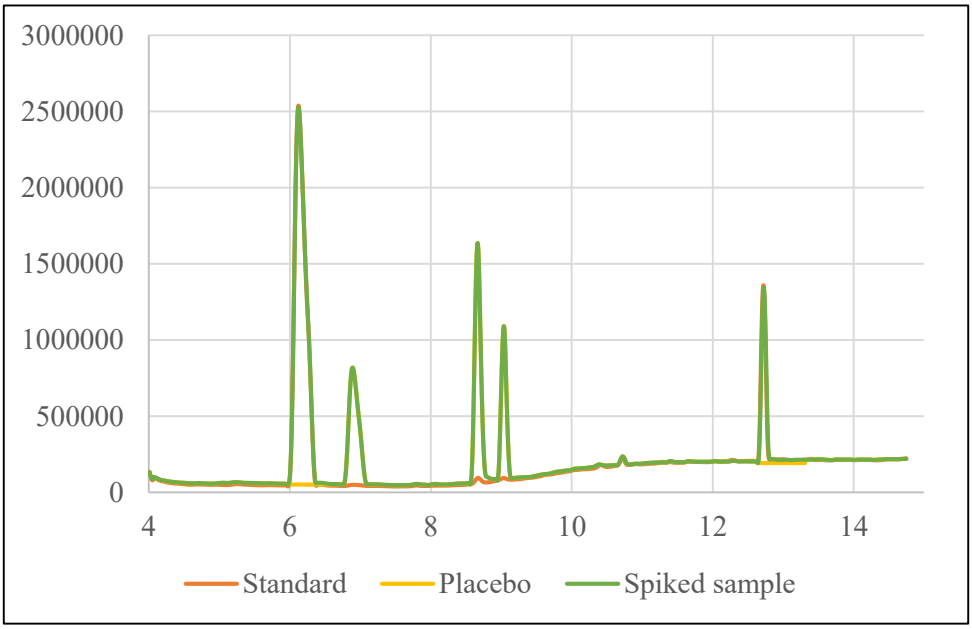


Figure 5. Chromatogram of solutions azelaic acid with internal standard (methyl docosanoate), placebo and spiked sample

3.4. Linearity

To view the level of linearity in the working range of the method according to the range of objective observation under the terms of the contents of analyte in the sample, the determination of linearity is required. This determination is carried out by making a spiked sample solution with at least five concentration levels, each of two replications in the working range of the method. The coefficient value of linear regression variance (Vx0) is calculated by the equation:

$$Vx0 = \frac{Sy/x}{b \,.\, x_{average}}$$

where b is slope and $X_{average}$ is the average content of azelaic acid in spiked sample solution.

The criteria for linearity acceptance is at a condition where the regression value of $r \geq 0.995$ and the value of $Vx0 < 5\%$. From the calculation it is obtained that value of $r = 0.9997$ (linear) for the range of content 63.44 µg/mL – 380.64 µg/mL as in the Figure 6 and Vx0 = 1.6%.

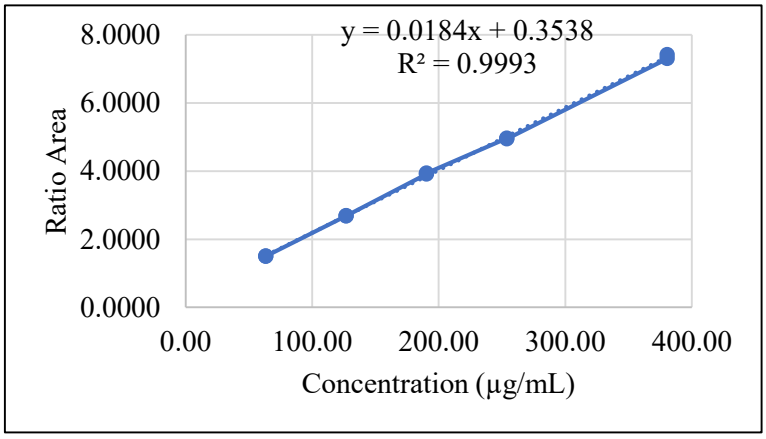


Figure 6. Linearity regression curve

3.5. Accuracy

Determination of accuracy is obtained by measuring the spiked sample solution at three levels of analyte concentration within the working range of the method (calibration curve). Each level consists of three spiked sample solutions.

The accuracy value is calculated based on the recovery value (%R), that is by the formula:

$$\%\ R = \frac{\text{Measurable content}}{\text{Actual content}} \times 100\ \%$$

It is obtained that the recovery value of azelaic acid in spiked sample solutions with a range of 2.5% - 7.5% is 98.27% -100.72%. Based on the requirements listed in [5], it meets the requirements, for which the analyte content in the sample ≥ 1% is 97-103%.

3.6. Precision

Precision determination was done by measuring the spiked sample solution at three levels of analyte concentration within the working range of the method (calibration curve). Repeat 3 times for each spiked sample solution, and calculated standard deviation (SD) and relative standard deviation (% RSD).

The results of this research showed that range of % RSD is 0.122% - 2.220%. Based on the requirements listed in [5], it meets the requirements, for which the analyte contents in the sample ≥ 1% RSD should be < 2.7%.

3.7. Detection Limit (LOD) and Quantitation Limit (LOQ)

The detection limit (LOD) and quantitation limit (LOQ) in this research were determined by using the calibration curve method of spiked sample solution with five concentration levels. The LOD and LOQ values are determined by the formula:

$$\text{LOD} = \frac{3 \,.\, S_a}{b} \quad \text{and} \quad \text{LOQ} = \frac{10 \,.\, S_a}{b}$$

S_a is standard deviation of intercept and b is slope.

From the research results, the method of analysis produces LOD equal to 0,02% and LOQ equal to 0,07%.

4. Conclusions

The validation of azelaic acid analysis method by derivatization and GCMS analysis developed in this study provides good results. Therefore, this method can be adopted as a routine analysis in the laboratory.

Acknowledgment

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