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Optimization of Experimental Procedure for Determining Azelaic Acid in Cosmetics by Gas Chromatography Derivatized through Ethanol

Jinmei Yang, Qijin Geng, Yaobin Zhou, Yuanfang Wang, Zhihua Li, and Ying Liu*

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ABSTRACT: The quantitative evaluation of azelaic acid is becoming critical in the development of new medicinal products and in environment. A feasible method for the determination of azelaic acid in cosmetics by gas chromatographic-mass spectrometer detector (GC-MS) with derivation was developed and optimized. The derivative effect was good, when azelaic acid was derivatized through ethanol at room temperature for 10 min with 800 μL of sulfuric acid as a catalyst. A good linear relationship of azelaic acid derivative was present from 10 to 1000 mg L^{-1} (R^2 = 0.9997). Detection limit and quantitative limit of GC was 15 and 50 mg kg^{-1}, respectively. The recovery rate was in the range from 87.7% to 101% with all relative standard deviation (RSD) values less than 4%, denoting the method meeting the requirement of the analysis. Therefore, this method has the advantages of strong anti-interference ability and accurate results. Among the eight samples nominally azelaic acid, only three were detected. The respective content was 78 133, 16 710, and 2431 mg kg^{-1}. The results showed that the actual addition of the azelaic acid in the market was quite different with label identification, being worthy of further attention. Further, it also provided a favorable experience for the monitoring of azelaic acid in the environment.
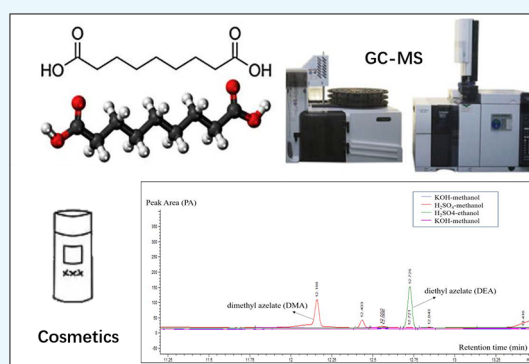
1. INTRODUCTION

Azelaic acid (heptane-1,7-dicarboxylic acid), a naturally occurring C9 dicarboxylic acid, is an important medium-long-chain dibasic acid, possessing significant biologic properties and a potential as a therapeutic agent. Because of its anti-inflammatory, antibacterial, and antikeratinization effects, it has significant effects in the treatment of acne,[1,2] melanoma,[3] rosacea,[4] cutaneous hyperpigmentation disorders,[5,6] and it blocks the formation of melanin and prevents spot formation.[7−9] In addition, as a plant inducer, it can induce tobacco disease resistance and improve plant resistance to pathogens. Additionally, azelaic acid is widely used in industry as a plasticizer and in chemical synthesis and also in producing spices and lubricants. It has an increasing demand in pharmaceuticals and cosmetics. However, excessive inhalation or intake of azelaic acid is harmful to the body and can cause water and air pollution.[10]

Fine aerosol particles (PM$_{2.5}$, < 2.5um in aerodynamic diameter) usually contain inorganic substances and hundreds of organic compounds such as dicarboxylic acids (DCAs).[10,11] In which, organic aerosols typically contribute 20−50%.[12] Low-molecular-weight C2−C9 DCAs in aerosols have received increasing attention. Because of the water-solubility and hygroscopicity, DCAs play an important role in atmospheric chemistry through atmospheric processing (e.g., secondary aerosol formation) and in the Earth's climate by enhancing the

ability of organic aerosols to act as cloud condensation nuclei.[13−15] In DCAs, azelaic acid exhibits high concentrations, for examle, at least 1.4 times higher than DCAs containing more than five carbons in marine and urban atmospheric particulates.[16] Further, in Ren's research, the characteristics and source apportionment of PM$_{2.5}$-bound carboxylic acids in Shanghai were investigated, by analyzing the adipic acid (C6)/azelaic acid (C9) ratio. A lower ratio of C6/C9 indicated that aerosols in Shanghai were more influenced by biogenic sources.[17]

Accordingly, the quantitative evaluation of azelaic acid is becoming critical in the development of new medicinal products and in the environment.[18] So far, the main detection methods include volumetric analysis,[19] gas chromatography (GC), modified GC using a mass spectrometer detector (GC-MS), and high-performance liquid chromatography with evaporative light-scattering detector (HPLC-ELSD).[20,21] Malik and Kaur developed a simple, rapid, and stable method for the analysis of
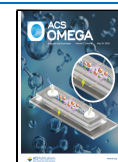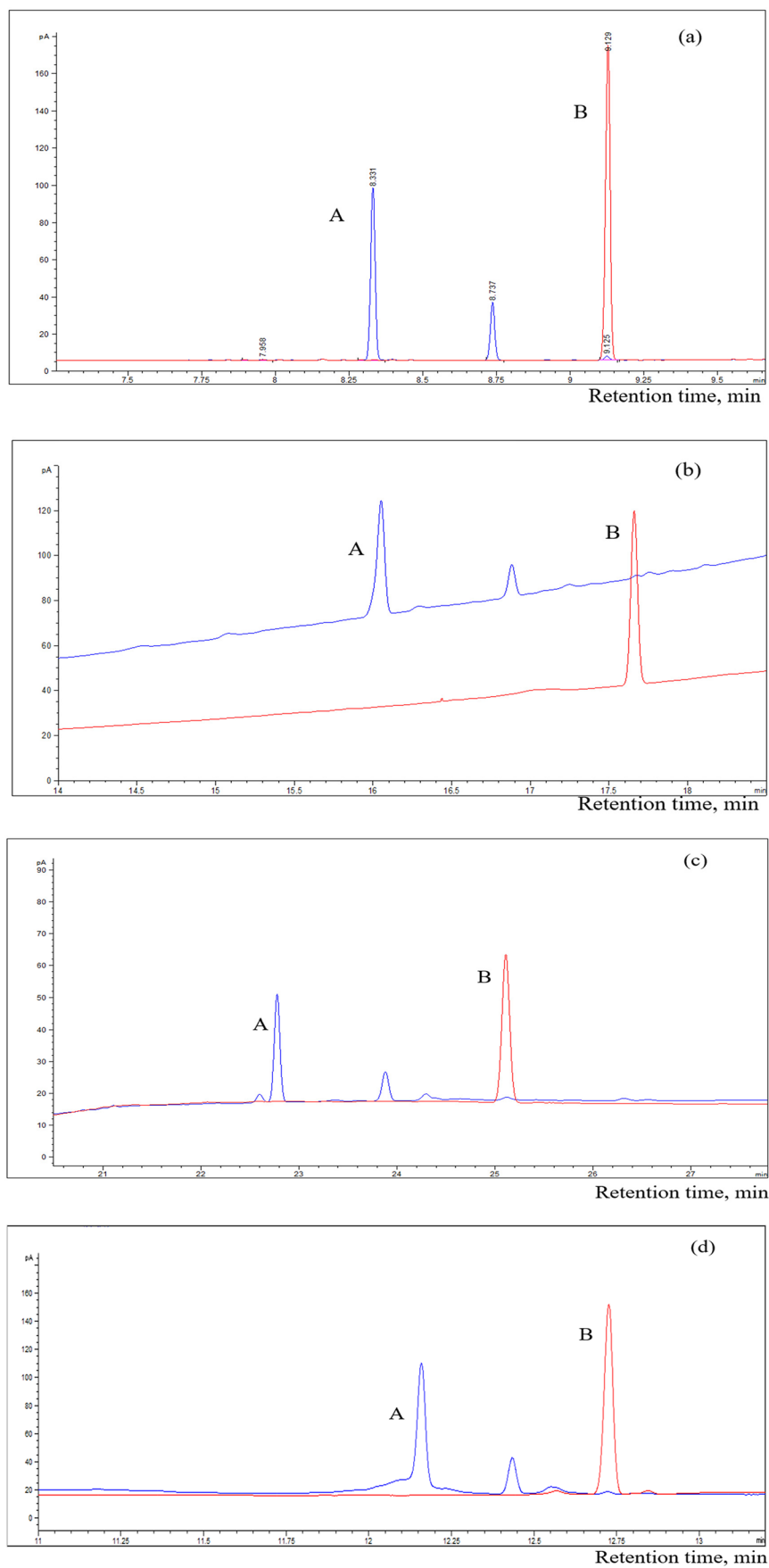
Figure 1. Gas chromatogram of azelaic acid derivatives by different separation columns (a) HP-5 (30 m × 0.25 mm × 0.25 μm); (b) DB-1 column (60 m × 0.53 mm × 1.0 μm); (c) DB-624 column (60 m × 320 μm × 1.8 μm); (d) CP-WAX column (50 m × 0.25 mm × 0.2 μm).

azelaic acid in cosmeceuticals by reversed-phase HPLC.[22] The interference of volumetric analysis is relatively large, it cannot be accurately determined. The ion chromatography (IC) method for DCAs had high efficiency but can only measure C2−C5 dicarboxylic acids. The physical chemical properties of azelaic acids are more suitable for detection by GC rather than liquid chromatography (LC). Though complicated and tedious, the GC-MS method for the determination of DCAs was sensitive.[23]

Several methods of azelaic acid derivatization by using chromatographic reagents have been developed[24] by derivatives with 2,4-dinitrophenylhydrazine, 2-bromoacetyl-6-methoxy-naphthalene, 1-leucine-4-methyl-7-coumarinylamide, phenacyl bromide, phenacyl esters, and tosylic acid. Palassis analyzed air samples of azelaic acid by GC detection derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA)-1% trimethyl-chlorosilane (TMCS).[25] Pusvaskiene et al. evaluated the content of azelaic acid in the natural aquatic environment by GC method using the ethylchloroformate as a reagent for derivatization.[26] Some studies demonstrated that the methyl derivatives were more popular because of its well-established literature procedures, inexpensiveness, and lower toxicity than others.[27] GC with the derivatization of fatty acids to methyl esters was used to determine the amount of azelaic acid in the oil paintings of old Flemish master painters,[28] the composition of Medieval and Renaissance Florentine paintings that originally contained azelaic acid,[29] and the content of azelaic acid in air[30,31] and in tobacco leaves.[32] Lusianti et al. developed a method to analyze azelaic acid in self-made cosmetics derivatized through 4 mL of BF_3-methanol 10% at 60 °C for 10 min.[27] Garelnabi et al. determined azelaic acids in a biological sample by GC, LCMS, and GCMS. Additionally, in GC analysis, 1 mL of 14% BF_3-methanol was used for the methylesterfication.[33] In the current literatures, lots of the derivation happened at a certain temperature. Few studies have discussed the optimization of sample treatment procedure before GC analysis. Further, BF_3 is highly reactive and easily explodes in water to produce a virulent fluoride smoke. Compared with methyl esters, ethyl ester derivatized through ethyl derivative is less toxic.

As a result, this research aimed to develop a feasible method for the determination of azelaic acid by derivation of ethyl derivative and optimize experimental procedure for the improvement of the monitoring methods. Further, the effectiveness of the analytical method is verified by the determination of actual samples. For the past few years, azelaic acid is increasingly used in pharmaceuticals and cosmetics because of its significant efficacy and relatively safety. Surveillance of such ingredient in products is hard to do. Therefore, attempts have been to establish and improve the measurement method of azelaic acids in cosmetics.

2. RESULTS AND DISCUSSION

2.1. Confirmation of Positive Sample by Mass Spectrum.

If necessary, mass spectrometry can be performed for positive samples, and the recommended conditions are as follows:

(a) Column: HP-5 ms (30m × 0.25 mm × 0.25 μm);
(b) Temperature-programmed: 80 °C (keep for 2 min), increased to 250 °C at 20 °C min^{-1} and kept for 6 min;
(c) Inlet temperature: 260 °C;
(d) Diversion ratio: 50:1;
(e) Ionization method: EI (electron ionization);
(f) Temperature of MS detector: 230 °C;

(g) Temperature of transmission line: 280 °C;
(h) Scan ion range: 50−550 amu;
(i) Carrier gas: helium (1.0 mL min^{-1}).

A MS analysis was conducted to compare azelaic acid derivative and DEA standard. Total ion flow diagram and ion fragmentation diagram (IFD) of two materials were obtained. By comparing the retention time of the standard peak and IFD, it was confirmed that the product derived from azelaic acid was diethyl azelate. The total ion flowchart and mass spectrum are listed in Appendix Figure 9 and and Figure 10.

2.2. Selection of GC Column.

The derivatives of azelaic acid by methanol-sulfate and ethanol-sulfate were confirmed through mass spectrum (MS) by matching to the reference spectrum. The derivatized sample solution was separated and detected on GC columns with different polarities. Four kinds of columns as HP-5 (30 m × 0.25 mm × 0.25 μm), DB-1 (60 m × 0.53 mm × 1.0 μm), DB-624 (60 m × 320 μm × 1.8 μm), CP-WAX (50 m × 0.25 mm × 0.2 μm) were selected to separate target compound. The gas chromatograms for each separated column were shown in Figure 1a−d. The abscissa is the retention time, and the ordinate is the peak area (PA). In the figures, the blue peak (A) is the azelaic acid-methanol derivative (DMA), and the red peak (B) is the azelaic acid-ethanol derivative (DEA).

Table 1 listed the parameters of DEZ on four kinds of column. Combined with Figure 1, HP-5 (30 m × 0.25 mm × 0.25 μm)

Table 1. Peak Wide and Symmetrical Factor of Chromatographic Peak

column	HP-5	DB-1	DB-624	CP-WAX
peak wide	0.0181	0.0489	0.0863	0.0411
symmetrical factor	1.12	0.981	0.998	1.635

was finally selected to be separation column because of the smaller peak width and better symmetry.

2.3. Selection of Derivative Conditions.

2.3.1. Selection of Derivative Reagents. The melting point of azelaic acid is from 98 to 103 °C and the boiling point is 286 °C (100 mmHg). Therefore, in theory, azelaic acid can be directly detected with GC. The standard of azelaic acid was dissolved with ethanol and diluted to 200, 400, 600, 800, and 1000 mg L^{-1}. These solutions were analyzed to obtain a calibration curve of azelaic acid (Appendix Figure 11). However, the intercept of the standard curve was too large. It might be caused by the molecular polymerization of azelaic acid during temperature programming of the column, resulting in azelaic acid with a low concentration unable to be detected. The results confirmed the separation of unmodified azelaic acid is rather problematic because the compounds are polar, low-volatility, and tend to adhere to the walls of GC columns.[34] Therefore, derivatization procedures were required before GC analysis to increase the volatility and improve separation.

Considering that there are two carboxyl groups of azelaic acid, the ester derivative is more stable and easier to detect.[27] It is thus feasible to use an esterification reagent or an acylation reagent to convert carboxyl group to ester. Because of the presence of a water phase in cosmetics, boron trifluoride-ethyl ether complex was not suitable as an acylation reagent. However, sulfuric acid methanol (methanol-sulfuric), sulfuric acid ethanol (ethanol-sulfuric), potassium hydroxide methanol (KOH-methanol), and potassium hydroxide ethanol (KOH-ethanol) were alternatives as esterifying derivative reagents. The spectrum of derivatives
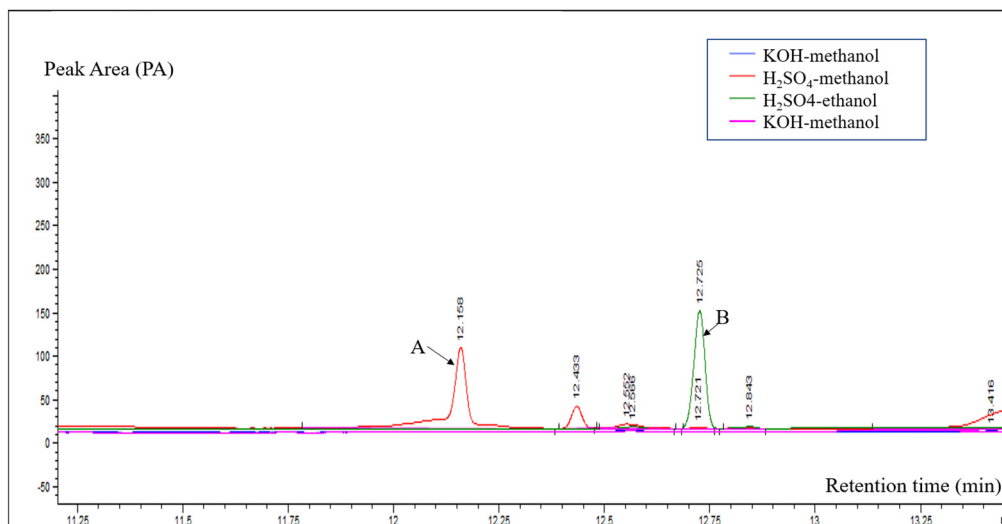
Figure 2. Chromatogram of derivatives based on different derivative reagents.

was produced by adding 200 mg L^{-1} of standard azelaic acid in a blank sample under the same condition. The normalized derivative spectra of different derivative reagents are shown in Figure 2, in which the Y-axis was the peak response represented by PA.

In the figure, the red peak (A) was a derivative product obtained from methanol-sulfate as a derivative reagent and confirmed as DMA by MS. The green peak (B) was a derivative obtained through ethanol-sulfate, and MS confirmed the derivative as DEA. There was no derivative by using KOH-ethanol and KOH-ethanol as derivative reagents. The esterification reaction is the reaction between an azelaic acid with an alcohol solvent which required an acid catalyst to produce an ester. Ethanol-sulfate was selected as the better derivative reagent because of the larger peak area of derivative, higher response value, higher sensitivity, and lower toxicity of ethanol under the same condition. Meanwhile, there was no azelaic acid ester confirmed by MS, before and after the ethanol-sulfate derivative.

2.3.2. Dosage Optimization of Derivative Reagent. The effects of different amount of concentrated sulfuric acid (50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 μL) on the derivative content were also investigated. The same volume standard solution of azelaic acid was respectively added into 1 g of sample, and then 2 mL of ethanol and different volume of concentrated sulfuric acid was added for derivation. Each amount was repeated three times (n = 3). Figure 3 shows the results depicted with respective standard deviation (SD).

The derivative content was gradually increased as the amount of concentrated sulfuric acid increased. When the amount added was 800−1200 μL, the derivative concentration reached the largest. Then, when the sulfuric acid was further increased, the concentration of derivative decreased gradually. Therefore, it was better to conduct subsequent experiments using 800 μL of sulfuric acid.

2.3.3. Optimization of Derivative Temperature. The effects of different derivative temperature (room temperature and 30, 40, 50, 60, 70, and 80 °C) on the derivative process were also investigated. A 1 g of sample was added into the same volume standard solution of azelaic acid, and then 2 mL of ethanol and 800 μL of sulfuric acid were added for the derivation. Each
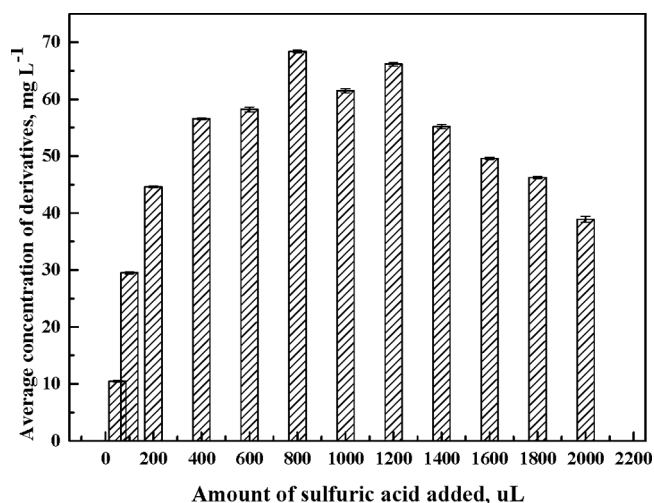

Figure 3. Change trend of derivative content with different amounts of sulfuric acid.

amount was repeated three times (n = 3). The results with respective SD are shown in Figure 4.

The content of the derivative changed little within the range from room temperature to 40 °C. The average derivative content was in the range of 65−69 mg L^{-1}. Then, the derivative concentration gradually decreased with the increasing temperature. When the temperature changed from 70 to 80 °C, the derivative content did not change significantly as before. It is known that a large amount of heat is generated because of the addition of concentrated sulfuric acid. The derivative reaction can be completely achieved at room temperature. Therefore, the derivative reaction temperature was set as room temperature.

2.3.4. Optimization of Derivative Time. The effects of different reaction time (0, 10, 20, 30, 40, 50, and 60 min) on the derivative process were also investigated. The same experimental process with section 2.3.3 with different derivative time was carried out, as shown in Figure 5.

It can be seen that the derivative concentration was basically constant within 0 to 30 min. However, the content slightly decreased from 30 to 60 min. In the experiment, the average concentration changed in the range of 60.4−71.1 mg L^{-1}, suggesting that the reaction time had little effect on the
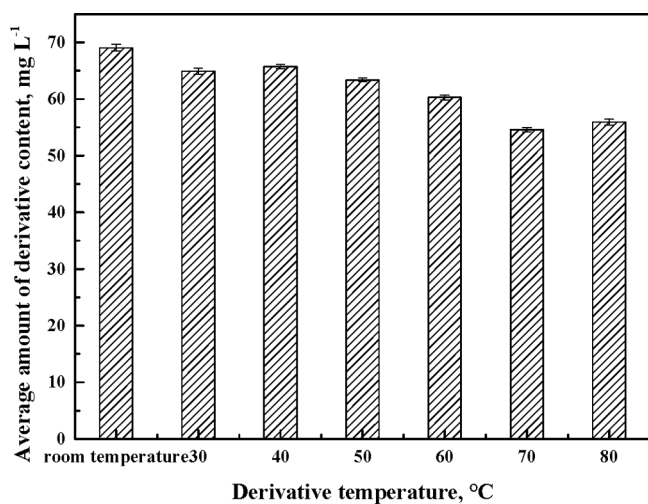
Figure 4. Change trend of average derivative content with derivative temperature.
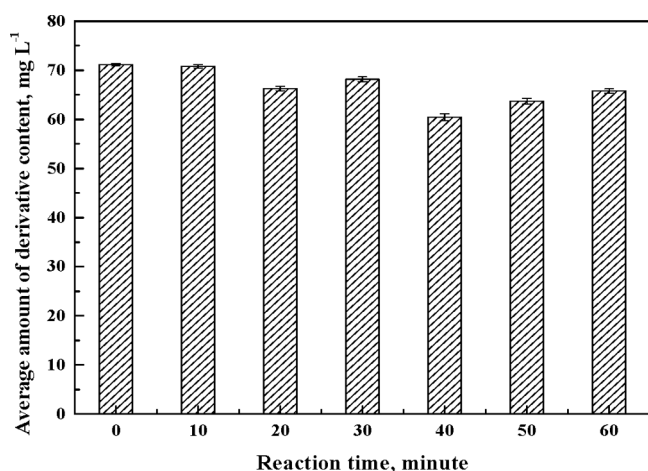

Figure 5. Change trend of derivative content with reaction time.

derivative reaction. The derivative reaction was basically completed when the derivative time was 10 min.

2.4. Optimization of Pretreated Conditions. *2.4.1. Selection of Extraction Solvent.* According to the water-insoluble nature of the derivative, *n*-hexane, ethyl acetate, toluene, and

acetonitrile commonly were selected as extraction solvents. In 1 g of sample, the same volume of azelaic acid standard solution, 2 mL of ethanol, and 800 μL of concentrated sulfuric acid were successively added. Then the solution was vortex mixed and derived for 10 min. Next, the same volume of *n*-hexane, ethyl acetate, toluene and acetonitrile was respectively added in the solution to extract derivative. Three times were repeated for each extraction solvent. The acetonitrile was found to be completely mutually solved with the derived solution and could not be used as extraction solvent. Average derivative concentration with extraction solvents of *n*-hexane (A), ethyl acetate (B) and toluene (C) was 105.4 mg L^{-1} (1.552), 83.1 mg L^{-1} (2.554) and 80.7 mg L^{-1} (2.211), respectively. In which, the numbers in parentheses represented corresponding SD value. All SD values were lower than 3. Derivative chromatogram with different extraction solvents was shown in Figure 6.

It can be seen that *n*-hexane (blue line, A) was the best extraction solvent among three ones, with the highest extraction efficiency and maximum extract derivative concentration. Meanwhile, the extraction solution was not easy to layered when ethyl acetate was used as an extracted solvent. In addition, when ethyl acetate and toluene were used as extracted solvents, there were more disturbing substances of the extraction solution spectrum of the sample matrix; while less disturbing substances existed when *n*-hexane as extracted solvent. Consequently, *n*-hexane was selected as extracted solvent in the subsequent experiment.

2.4.2. Selection of the Extraction Times. In 1 g of sample, we successively added the same volume standard solution of azelaic acid, 2 mL of ethanol, and 800 μL of concentrated sulfuric acid, and the sample was shaken vigorously for 10 min. After the derivatization reaction, the derivative was extracted with *n*-hexane for one, two, three, and four times, to investigate the effect of the number of extraction times on derivative results. Each group was repeated three times ($n = 3$). After the first, second, third, and fourth extraction, respectively, the average derivative concentration of each group with SD value in parentheses was 105.0 mg L^{-1} (4.496), 27.3 mg L^{-1} (2.170), 6.31 mg L^{-1} (0.224), and 6.83 mg L^{-1} (0.240). These results show that the concentrations of derivatives were gradually decreased as the number of extraction times increased. The derivative concentration was only 6.83 mg L^{-1} in the fourth extraction. Therefore, after extracting three times, the
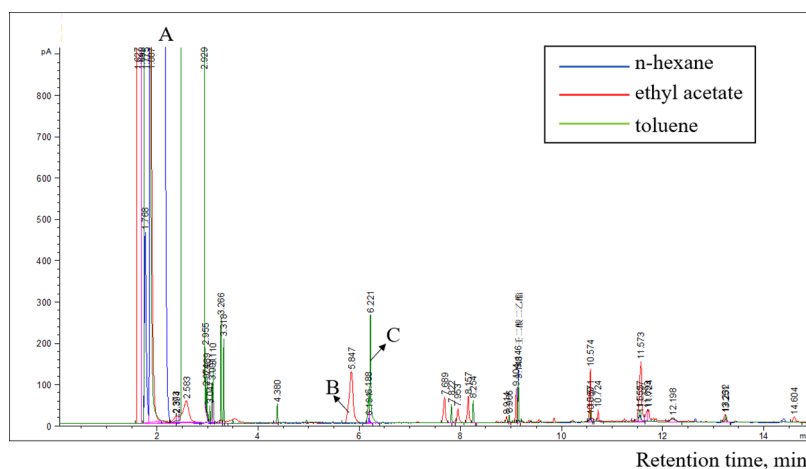

Figure 6. Chromatogram of derivate with different extraction solvents.

Table 2. Influence of Water on Derivative Reaction Process

simulated reagent matrix/1.0 mL	ethanol	water:ethanol = 1:3	water: ethanol = 1:1	water:ethanol = 3:1	water
the average of actual derivative content of azelaic acid/mg L^{-1}	242	240	216	215	171
recovery rate %	96.80%	96.00%	86.60%	86.20%	68.40%

Table 3. Derivative Yield of Ethanol (a) and Ethanol Containing 75% Water (b)

pure DEA concentration/mg L^{-1}	peak area of DEA	peak area of derivative (a)	yield (a)/%	peak area of derivative (b)	yield (b)/%
10	31.37	37.71	120.2%	35.66	113.7%
20	63.99	74.10	115.8%	69.15	108.1%
50	172.26	187.67	108.9%	176.93	102.7%
100	346.10	383.23	110.7%	365.80	105.7%
200	649.73	799.74	123.1%	738.54	113.7%
500	1654.22	1848.33	111.7%	1765.02	106.7%
1000	3440.07	3722.50	108.2%	3539.26	102.9%

concentration of derivatives was basically unchanged, and the extraction times was set as three.

2.4.3. Selection of Purification Condition. A small amount of sulfuric acid remained in the extracted solution after extracted with *n*-hexane, so it was necessary to purify the extracted solution to remove residual sulfuric acid. Considering the properties of derivatives and sulfuric acid, deionized water, saturated sodium bicarbonate solution (NaHCO$_3$), and sodium hydroxide solution (NaOH) of 20 g L^{-1} were selected as purifying solution to reveal the influence of purification conditions on the results. Measurements of derivative content for each purifying solution were repeated three times. The average derivative concentrations for three kinds of samples, with corresponding SD value in parentheses, were 93.7 mg L^{-1} (0.058), 102.5 mg L^{-1} (0.513), and 100.7 mg L^{-1} (5.783).

The contents of the derivative were different, while the MS spectra did not show obvious change with different purification reagents. Therefore, saturated sodium bicarbonate was selected as a purification agent.

2.5. Influences of Water on Derivative Reaction Conversion. The effect of water on the derivation process should be considered, as almost all cosmetics contain water. Five kinds of simulated reagent matrix, including ethanol, water:-ethanol (1:3, v/v), water:ethanol (1:1, v/v), water:ethanol (3:1, v/v), and water were selected to optimize the experiment conditions. Then 0.125 mL of an azelaic acid stock solution with a concentration of 10 mg mL^{-1} was added respectively into 1 mL of each reagent matrix to derive according to standard methods. Determinations of derivative concentration for each sample were repeated twice. The theoretical derivative content of azelaic acid was 250.0 mg L^{-1}. The content of diethyl derivative in each sample was investigated, as shown in Table 2. In which, the recovery rate referred to the ratio of the actual derivative content of azelaic acid to the theoretical value.

It could be confirmed that under the same derivative conditions, the recovery rates were still higher than 80% until the water content increased to 75% (water:ethanol = 3:1). The general water content of the commercially available cosmetics containing azelaic acid is less than 75%. Therefore, the water content of the sample cannot affect the yield of the derivative under the standard method.

Meanwhile, the research compared the derivative calibration curves obtained from two kinds of derivative reagent, with the concentration range of DEA being 10−1000 mg L^{-1}. One was derivatization from ethanol (a), and another was produced by ethanol + water (1 + 3) (b). The derivative efficiency of two

cases expressed in yield was listed in Table 3. In which, yield denoted the ratio of PA of derivative to PA of pure DEA.

As can be seen from the table, compared with pure DEA standard, the derivatization efficiency was 102.7%−123.1%, whether it used a pure organic solvent or contained 75% water as derivatives. There was no significant impact on the test results by derivatization of azelaic acid standards.

2.6. Methodology Investigation. *2.6.1. Standard Curve and Linear Range.* The standard solution of azelaic acid was derived to obtain a standard working curve with the concentration range of 10−1000 mg L^{-1}. Figure 7 depicted
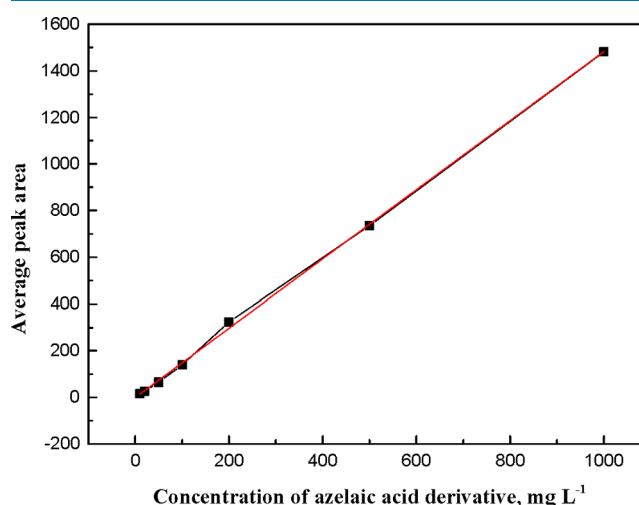
Figure 7. Standard curve of azelaic acid derivatives

the standard curve with the concentration of azelaic acid derivative as the abscissa, and the corresponding average PA as the ordinate. Calibration curve could be expressed as PA = 1.483 × retention time − 0.0745. The curve displayed good fitness in the concentration range of 10−1000 mg L^{-1}, and the linear regression R^2 value was about 0.9997 (adjusted R^2 = 0.9994). It indicated that the standard curve was available in the statistical testing.

2.6.2. Detection Limit (LOD) and Quantitation Limit (LOQ). The signal-to-noise ratios of 10 injections were measured under the same conditions (n = 10). The LOD of method was calculated to be 15 mg kg^{-1} (15 ppm), and the LOQ equaled to 50 mg kg^{-1} (50 ppm).

2.6.3. Precision and Accuracy. Precision determination was carried out by measuring the spiked sample solution at three

levels of standard solution in the blank matrix (1, 2, and 10 times of LOQ). Each measurement was repeated seven times for each level ($n = 7$). SD and relative standard deviation (%RSD) were calculated. The results showed that all RSD values were within 4%, indicating that the precision of the method met the requirements of the analysis (Table 4).

Table 4. Precision Determination of Spiked Sample Solutions at Different Levels ($n = 7$)

added amount of azelaic acid/mg kg^{-1}	measured average amount/mg kg^{-1}	SD	RSD/%
50	49.6	1.942	3.92
100	94.2	1.961	2.08
500	495.7	14.246	2.87

Determination of accuracy was obtained by measuring the spiked sample solution with three sample types. Each sample consists of three levels of analyte concentration, and measurements were repeated seven times ($n = 7$) for each level. An azelaic acid standard with different concentrations was added into each blank sample, and the recovery test was conducted, as listed in Table 5. The accuracy value was calculated on the basis

Table 5. Recovery Rates and Relative Standard Deviation (RSD) ($n = 7$)

sample types	theoretical amount of adding standard/mg kg^{-1}	average recovery of adding standard/mg kg^{-1}	average recovery rate/%	RSD/%
facial mask	200	176	87.8	2.22
	600	526	87.7	2.34
	800	807	101	3.65
toner	200	188	94.1	2.75
	600	570	94.9	2.02
	800	785	98.1	1.93
emulsion	200	182	91.1	2.82
	600	543	90.5	3.92
	800	776	97.0	1.13

of the recovery value (%R). As can be seen from the table, the recovery rate was in the range of 87.7%~101% with all RSD values being lower than 5%, denoting the results satisfied the accuracy requirements of the method in the addition level.

2.7. Determination of Actual Sample. In the research, 76 kinds of commercially available cosmetics were selected to carry out the test. In which, eight samples nominally contained azelaic acid; however, only three samples were detected, and the respective concentrations were 78 133, 16 710, and 2431 mg

kg^{-1}. To further examine the reliability of the method, the samples whose labels indicated they contained azelaic acid but it remained undetected were selected for further experiment (five kinds of samples). Recovery rate was examined by adding three levels with different concentrations of azelaic acid (A: 50 mg kg^{-1}; B: concentration at normal addition of product (2.5%); C: concentration of the intermediate point (750 mg kg^{-1}). The experiment was repeated twice for each spiked sample solution. Sample names were represented by sample A, sample B, and so on, and the results are listed in Table 6.

All recovery rates were in the range of 83.6%−109.5% with the average rate of 97.39%. When the amount of adding standard solution was 50, 750, and 2500 mg kg^{-1}, the average rate was 101.0%, 101.3%, and 89.9%, respectively. The results showed high recovery rates, demonstrating the results were credible.

3. EXPERIMENTAL SECTION

3.1. Reagents and Instruments. An Agilent 7890N GC with a FID detector, USA Agilent Instrument Co., Ltd. was used. Reagents (AR) were purchased from National Pharmaceutical Group Chemical Reagent Co., Ltd. Azelaic acid standard was purchased from Shanghai Pu Yu Technology Co., Ltd.

3.2. Working Conditions of the Instrument.

(a) column (DB-1, 30 m × 0.250 mm, 0.25 μm)
(b) carrier gas: N$_2$ (1.0 mL min^{-1})
(c) inlet temperature: 260 °C
(d) injection volume: 1 μL
(e) diversion ratio: 5:1
(f) FID detector: temperature 280 °C, hydrogen flow 30 mL min^{-1}, air flow 300 mL min^{-1}
(g) Temperature programming was divided into four phases. The initial temperature was 60 °C for the first 2 min, increased to 150 °C at 10 °C min^{-1} and keep for 1 min, raised to 165 °C at 5 °C min^{-1} and maintained 2 min, then reached to 250 °C at 25 °C min^{-1}.

3.3. Pretreatment Method of Sample. A 1 g sample was accurately weighed (~0.001g) and added into a 15 mL scale tube, treated with 2 mL ethanol without methanol, and shaken vigorously for 1 min. Then 0.8 mL of concentrated sulfuric acid was added and vortex mixed for 1 min. The final liquid was the derivative after it remained for 10 min at room temperature.

n-Hexane (5 mL) was added to the derivative and shaken vigorously for 2 min. After the solution was centrifuged at 5000 revolutions min^{-1} for 5 min, the layer of *n*-hexane was sucked into the 50 mL scale tube. The above operation was repeated twice to get all *n*-hexane extract. Afterward, the saturated sodium bicarbonate solution with equivalent volume was added dropwise to the *n*-hexane extract. The solution was centrifuged

Table 6. Recovery Rate of Samples Containing Azelaic Acid but Undetected

samples	sample A	sample B	sample C	sample D	sample E
original test results/mg kg^{-1}	<50	<50	<50	<50	<50
theoretical amount of adding standard/mg kg^{-1}			50		
average concentration of sample content/mg kg^{-1}	50.0	49.8	51.3	50.2	51.3
recovery rate/%	99.9%	99.6%	102.6%	100.4%	102.6%
theoretical amount of adding standard/mg kg^{-1}			750		
average concentration of sample content/mg kg^{-1}	715	746	782	733	821
recovery rate %	95.3%	99.5%	104.3%	97.7%	109.5%
theoretical amount of adding standard/mg kg^{-1}			2500		
average concentration of sample/mg kg^{-1}	2.21 × 10^3	2.09 × 10^3	2.40 × 10^3	2.24 × 10^3	2.29 × 10^3
recovery rate/%	88.6%	83.6%	96.0%	89.7%	91.5%

Confirmation of positive sample by GC-MS
⇩
Selection of GC column
⇩
Optimization of derivative condition { **Derivative reagents** / **Dosage** / **Temperature** / **Time**
⇩
Optimization of pretreatment conditions { **Extraction solvent** / **Extraction times** / **Purification condition**
⇩
The influence of water
⇩
Methodology investigation { **Standard curve of derivative** / **LOD and LOQ** / **Precision and accuracy**
⇩
The determination of actual sample

Figure 8. Experimental procedure of entire research.

at 5000 revolutions min^{-1} for 5 min after it was oscillated with continuous venting. The *n*-hexane layer was sucked in a 15 mL scale, blown to about 3 mL by N$_2$ at room temperature, and transferred to a 5 mL volumetric flask. The 15 mL scale tube was washed with a small amount *n*-hexane. The solution was combined in the same 5 mL volumetric flask, and set the volume to the mark. This was the sample solution to be measured for the subsequent experiment.

3.4. Preparation of Standard Working Curve. First, 1 g of azelaic acid standard (0.0001 g) was accurately weighed and dissolved in a 100 mL volumetric flask with methanol-free ethanol, as a standard stock solution with a solution concentration of 10 mg mL^{-1}. Working standard solutions were obtained by diluting the stock solution to 50, 100, 250, 500, 1000, 2500, and 5000 mg L^{-1} with methanol-free ethanol.

Initially, 1 mL of each standard working solution was accurately taken and combined with 2 mL of methanol-free ethanol. Then a series of sample processing including

derivatization, extraction, nitrogen blowing, and constant volume was conducted. Afterward, new derived working standard solutions (10, 20, 50, 100, 200, 500, and 1000 mg L^{-1}) were obtained according to the treatment method of the samples.

A 2 g of sample was dissolved with ethanol to a final volume of 10 mL, and 2 mL of derivative reagent of concentrated sulfuric acid was added and shaken vigorously. The solution was derived using water bath at 70 °C for 1 h, rapid cooled to room temperature, and 5 mL of *n*-hexane was added. The upper layer was analyzed after centrifuging.

3.5. Method Validation. The linearity, LOD, LOQ, and precision were evaluated for the method of analysis. SD and RSD of samples were calculated.

Experimental procedure of the research was illustrated in Figure 8.

4. CONCLUSION

This study established the sample treatment method in cosmetic by derivatization of ethanol with sulfuric acid as a catalyst and optimized the experimental procedure. It effectively reduced the interference of impurities and confirmed the GC method for the determination of azelaic acid in cosmetics. Azelaic acid derivative displayed good fitness in the range from 10 to 1000 mg L^{-1} with a high R^2 (R^2 = 0.9997). LOD and LOQ values of the method were 15 and 50 mg kg^{-1} respectively. Recovery rate was 87.7%∼101% with RSD values within 5%, denoting precision and accuracy of the method meeting the requirements of the analysis. This method has the advantages of strong anti-interference ability and accurate results. It is suitable for regulatory authorities and manufacturers to monitor the content of azelaic acid in cosmetic products. It contributes to the labeling behavior of the product active ingredients in the market. Further, it also provides experience for the monitoring of C2−C9 organic acids in the environment.
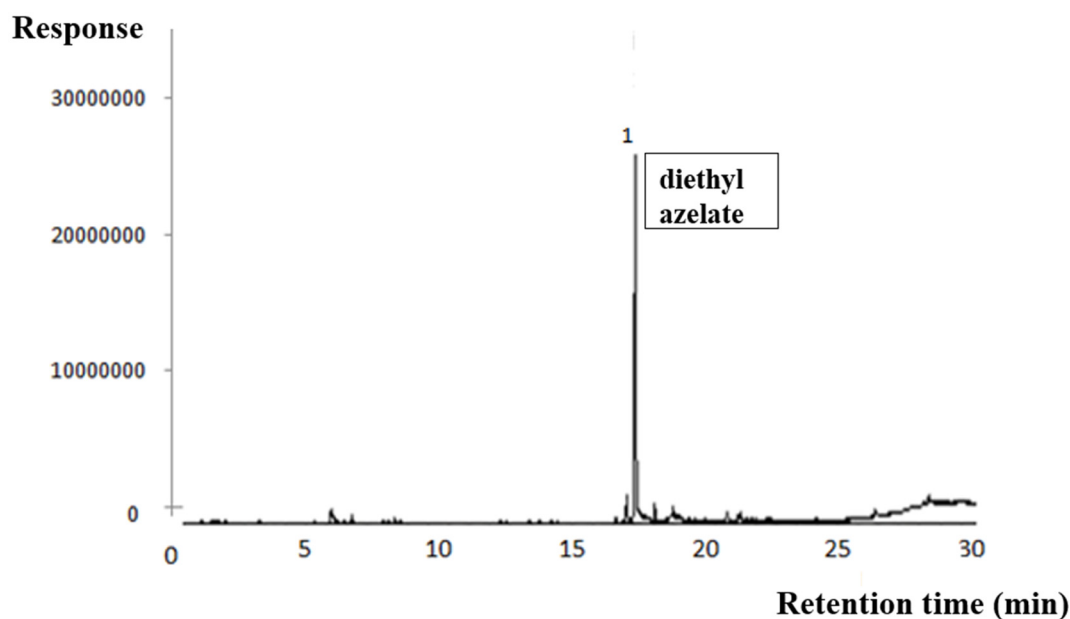
■ APPENDIX

The Appendix contains Figures 9−11

Figure 9. Total ion flowchart of diethyl azelate.
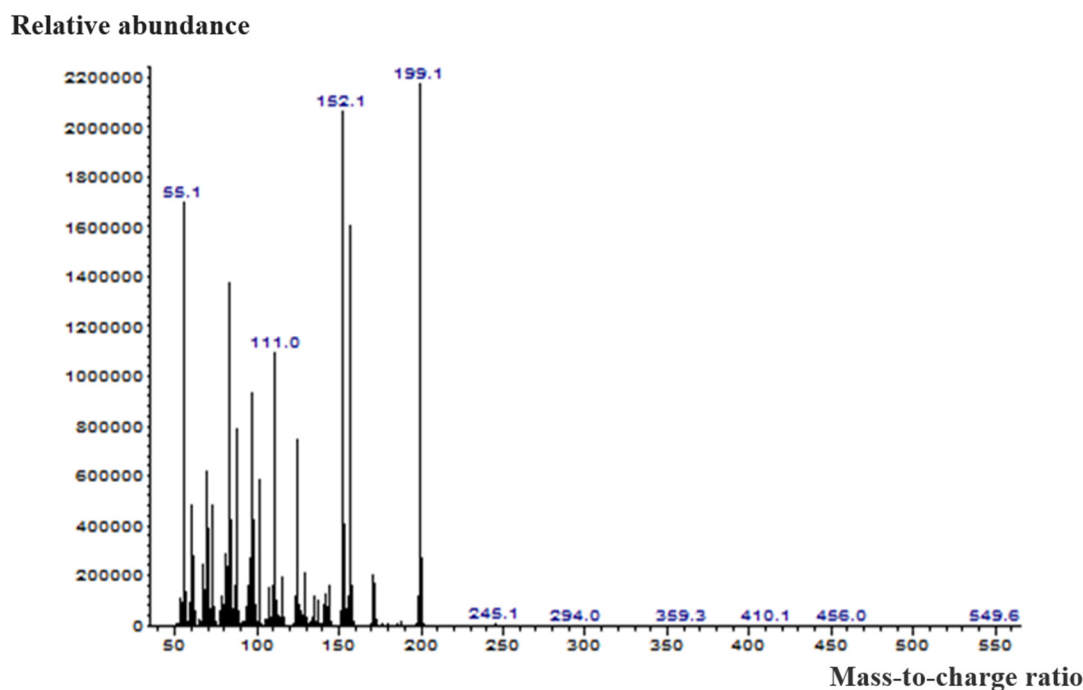
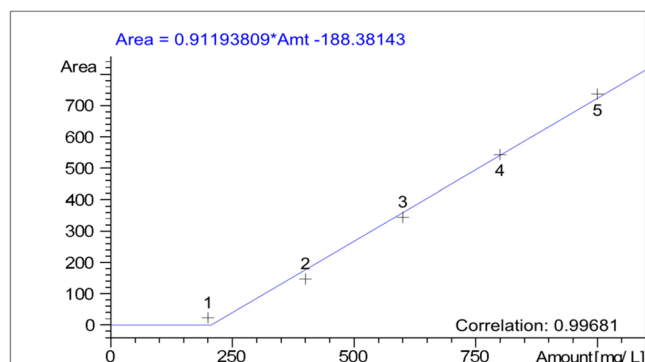**Figure 10.** Mass spectrometry of diethyl azelate.

Figure 11. Standard curve under nonderivative conditions.

■ AUTHOR INFORMATION

Corresponding Author

Ying Liu − *Department of Chemistry-Chemical & Environment Engineering, Weifang University, Weifang 261061, P. R. China*; Email: ydliuying@163.com

Authors

Jinmei Yang − *Department of Chemistry-Chemical & Environment Engineering, Weifang University, Weifang 261061, P. R. China; Environmental Pollution Control Institution of Weifang University, Weifang, Shandong 261061, China;* ⊚ orcid.org/0000-0003-1900-1815

Qijin Geng − *Department of Chemistry-Chemical & Environment Engineering, Weifang University, Weifang 261061, P. R. China; Environmental Pollution Control Institution of Weifang University, Weifang, Shandong 261061, China*

Yaobin Zhou − *Shanghai Institute of Quality Inspection and Technical Research, Shanghai 200233, China*

Yuanfang Wang − *Department of Chemistry-Chemical & Environment Engineering, Weifang University, Weifang 261061, P. R. China;* ⊚ orcid.org/0000-0002-0329-2649

Zhihua Li − *Weifang Water Quality Testing Co., Ltd, Weifang, Shandong 261061, China*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.2c00464

Notes

The authors declare no competing financial interest.

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