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Research Article

DEVELOPMENT OF A DIRECT METHOD OF ANALYZING TRANEXAMIC ACID LEVELS IN WHITENING CREAM USING REVERSED PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: Whitening cream is a cosmetic that contains ingredients that can alleviate hyperpigmentation. Tranexamic acid (TA) is one of the potential anti-pigmentation agents that work through inhibiting plasmin. TA is used in cosmetic formulations at a concentration of 2.5% as a whitening and moisturizing agent. To date, research on TA in both cosmetics and other pharmaceutical products using high-performance liquid chromatography (HPLC) has not been done directly (without derivatization). Therefore, this study aimed to develop a simple and rapid analytical method for TA (without derivatization) in cosmetic cream samples using reverse-phase HPLC and water as a solvent.

Methods: Optimization was conducted by evaluating several parameters that affect sample extraction, as well as composition and mobile phase types. The optimal method must fulfill suitability and validation requirements. The optimal method should be able to detect and quantify TA in cream samples without derivatization.

Results: The optimal analysis condition used a ultraviolet detector at a wavelength of 210 nm, acetonitrile: double-distilled water: phosphoric acid (64:34:2) as the mobile phase and a flow rate of 0.8 mL/min. The retention time of the analyte occurred in the 2nd min.

Conclusion: The analytical method that met the validation requirements was characterized using parameters such as accuracy, precision, linearity, selectivity, limit, of detection, and limit of quantitation. This method is applicable for analyzing TA content in samples with a concentration of 1.02%.

Keywords: Reverse-phase high-performance liquid chromatography, Optimization and validation, Tranexamic acid, Whitening cream.

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INTRODUCTION

Tranexamic acid (TA) (Fig. 1) is an antifibrinolytic agent used to treat menorrhagia. In addition, TA also has a whitening effect against hyperpigmentation caused by melasma and ultraviolet (UV) radiation [1]. TA has been studied for its anti-melasma potential compared with standard therapy [2]. These reports revealed that oral or topical TA is similarly effective as standard therapy in patients with melasma [3]. Some studies additionally stated that TA has greater efficacy with fewer side effects. TA has emerged as a promising treatment for melasma both alone and in combination with other treatments [4-6]. TA is used as a whitening and moisturizing agent in cosmetic formulations at a concentration of 2.5% [7]. According to Japanese regulations regarding products containing TA, a cosmetic product is considered safe as a whitening agent if its TA concentration does not exceed 1.5–2% [8]. TA can also cause severe irritation and allergies under skin conditions that are sensitive to the agent [9-11]. TA does not have a high number of chromophore groups, and thus it is difficult to detect through UV spectroscopy. Analyses of TA in pharmaceutical products through high-performance liquid chromatography (HPLC) always involve derivatization to obtain a higher number of chromophore groups. The previous studies on the derivatization of TA used derivative agents such as 0.2% ninhydrin in methanol [12], phenyl isothiocyanate [13], 2-hydroxynaphthaldehyde in ethanol [14], sodium picryl sulfonate [15], benzenesulfonyl chloride [16], and 2,4-dinitrofluorobenzene [17]. None of these studies reported direct analysis using UV-HPLC. Therefore, this study analyzed TA content in a cosmetic sample in the form of a cream without derivatization using reverse-phase HPLC. The method of sample preparation and HPLC analysis was optimized to increase its sensitivity and selectivity to permit TA analysis without derivatization through a simpler method.

METHODS

Instrumentation

An LC 20AT HPLC system (Shimadzu, Japan) was equipped with a pump, SunFire™ C_{18} column, SPD-10A UV-Vis detector (Shimadzu), manual injector, and data processor (LC-Solution). A UV-Vis spectrophotometer (Jasco V-530), HPLC syringe (SGE, Australia), centrifuge (Labofuge 5100), vortex (Thermo Scientific), micropipette (Eppendorf), Ultrasonic Sonicator, hotplate (IKA® C-MAG HS 7), pH meters (Eutech Instruments pH 510), and 0.45-µm Whatman filter membrane were also utilized.

Chemicals and reagents

TA (Hunan Dongting Pharmaceutical Co., Ltd.), HPLC grade acetonitrile (Merck), glacial acetic acid (Merck), ammonium acetate (Merck), double-distilled water (Ikapharmindo), potassium dihydrogen phosphate (Merck), methanol (Merck), and cream whitening samples were obtained from commercial suppliers.

Chromatographic conditions

Chromatographic separation was conducted using a C_{18} column as the stationary phase and acetonitrile: double-distilled water: phosphoric acid (64:34:2) v/v/v as the mobile phase at a flow rate of 0.8 mL/min. Chromatographic detection was performed using a UV-Vis detector at a wavelength of 210 nm.

Standard and working solution preparation

The standard stock solution of TA (1000 µg/mL) was prepared by dissolving 100 mg of TA in 70 mL of distilled water in a 100-mL volumetric flask. The solution was saturated for 15 min and solvent was added up to a volume of 100 mL. The working solution was prepared by diluting the stock solution with solvent to obtain 200 µg/mL TA.

Sample preparation

Extraction was performed by dissolving 150 mg of a cream sample in 10 mL of water and the mixture was heated at 100°C until the sample dissolved completely. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was separated and filtered through a 0.45-µm membrane filter. Then, 20 µL of the sample were injected and the chromatogram was recorded.

System suitability test

In total, 20 µL of 200 µg/mL TA were injected into the HPLC system under the optimal analysis conditions. The injection was repeated up to six times. The results of each trial were recorded and used to calculate the coefficient of variation (CV). The required CV was ≤2% [5].

Method validation

This method is validated using parameters such as selectivity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision.

Selectivity

Selectivity was examined by comparing the chromatogram of a blank cream solution with a standard solution around the retention time of TA. There should be no disturbance in the retention time of TA in the chromatogram of the blank solution.

Linearity

Linearity tests were performed using standard solutions at six concentrations over the range of 150–700 µg/mL. Each concentration was obtained by diluting the 1000 µg/mL standard solution. The calibration curve plotted the relationship between the concentration and area using the least square method.

LOD and LOQ

LOD and LOQ were calculated using a linear regression calibration curve and an Sb value equal to the residual standard deviation (S[y/x]).

Accuracy and precision

Accuracy and precision tests were conducted using simulated or spiked placebo recovery methods. The standard number for each concentration of 80, 100, and 120% (16, 20, and 24 mg, respectively) was weighed. At each concentration, a number of matrices were added until a weight of 1 g was reached, and the sample was then dissolved in a 10-mL volumetric flask followed by extraction as described in the sample preparation stage. Accuracy was calculated using the percentage of recovery (% recovery), and precision was calculated using the percentage of the relative standard deviation based on three injections at concentrations of 80 and 120% and six injections at a concentration of 100%.

RESULTS AND DISCUSSION

Wavelength optimization analysis

To determine the maximum wavelength of a compound, UV-1600 series spectrophotometers should be used in the wavelength range of 190–400 nm. Determination of the maximum wavelength is important before the start of the analysis to permit the maximum absorption of the compounds to be analyzed using the reverse-phase HPLC system, but TA does not have sufficient numbers of chromophore and auxochrome groups to permit direct detection using UV spectrophotometry. Therefore, in this study, optimization of HPLC was performed at wavelengths of 200, 205, and 210 nm. The results for the peak area and number of theoretical plates were greater at 210 nm than at the other wavelengths. Data from the selection of wavelength analysis of TA compounds are presented in Table 1.

Optimization and mobile phase composition

Mobile phase composition was optimized using three different mobile phases. Analysis of TA using a mobile phase consisting of acetonitrile: phosphate buffer pH 3.6 (35:65 v/v) produced an asymmetrical peak, which was not ideal because it was difficult to determine its area. The mobile phase of acetonitrile: double-distilled water: phosphate buffer (64:34:2) produced the best peak shape and constant. The peak was observed at 2 min, which indicated that the method requires a short run time. Contrarily, the mobile phase of methanol: buffer pH 4 (75:25) did not produce an analyte peak even after 15 min of analysis. The analytical process was terminated after 15 min because results were obtained with a faster retention time using acetonitrile: double-distilled water: phosphate buffer (64:34:2) as the mobile phase. Therefore, acetonitrile: double-distilled water: phosphate buffer was selected as the mobile phase because it provided the best area results, retention time, and peak among the mobile phase combinations examined. The chromatogram of the mobile phase optimization is presented in Fig. 2.

Optimization of the flow rate

To further optimize the conditions, three different flow rates, namely, 0.8, 1, and 1.2 mL/min, were compared. The three flow rates resulted in retention times of 2.151, 1.727, and 1.435 min, respectively, and peak areas of 103 629, 82 545, and 103 629 µV/s, respectively.

The retention time decreased as the flow rate increased, whereas the area tended to become smaller because the separation did not occur perfectly. In addition, the pressure in the column also increased up to 100 kgf/cm^2 as the flow rate was increased. In this study, the optimal flow rate was 0.8 mL/min because it provided better resolution, a larger area, a large number of theoretical plates, a safe pressure

Table 1: Wavelength analysis results

Wavelength	Area (µV/s)	Number of theoretical plates
200 nm	103 629	2920
	93 367	3042
205 nm	110 600	3048
	101 788	3068
210 nm	113 055	3171
	128 553	2975

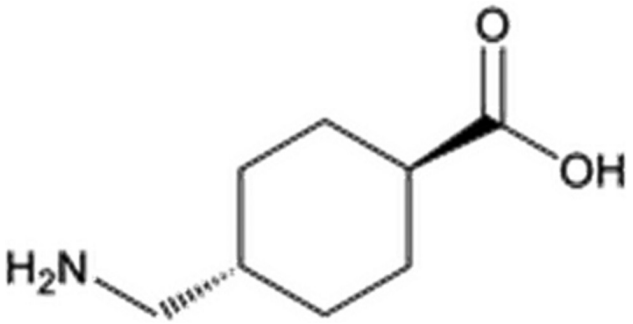

Fig. 1: Structure of tranexamic acid [6]

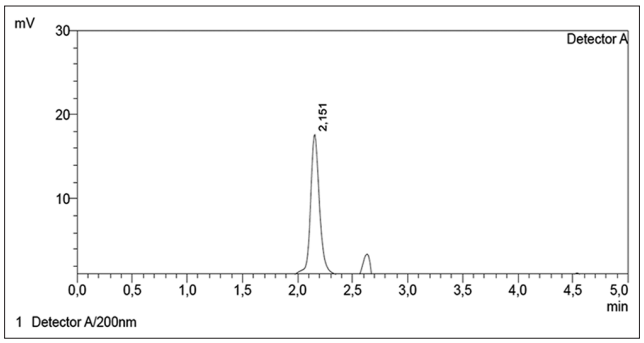

Fig. 2: Chromatogram of the standard solution of tranexamic acid

(70–90 kgf/cm^2), and a small height equivalent of a theoretical plate (HETP). Chromatograms and data from the selection of flow rates for TA compounds are presented in Table 2.

System suitability test

Before choosing the optimal analysis conditions, it is important to first perform a system suitability test because there might be differences in the type of equipment and techniques used. The following results were obtained after six repeated injections: HETP, 47.515; follow-up factor, 1304; CV, 1.667%; and number of theoretical plates, 3157. The obtained data met the requirements of the system suitability test because the CV was <2%. Data from the complete system suitability test are shown in Table 3.

Method validation

Selectivity

Selectivity was evaluated using the chromatograms of blank, standard, and sample solutions. The results did not reveal any interference of the retention time for TA compounds, which was 2.145 min. Injecting 20.0 µL of the placebo solution (cream matrix) also resulted in no interference of the retention time of TA. In the placebo chromatogram (cream matrix), there were peaks at 0.7 and 1 min, which were considered to represent other compounds present in the placebo. However, no other peaks were observed in the chromatogram of the placebo solution (cream matrix). This illustrated that the analytical method was selective for TA compounds. The placebo solution chromatogram (cream matrix) is presented in Fig. 3.

Linearity

A linear regression equation was obtained using six concentrations of standard solutions over the range of 150–700 µg/mL, namely y=543.78x−20 537 with a correlation coefficient of 0.99915. From the results of the analysis, it can be concluded that the calibration curve met the linearity test requirements. Data from the linear regression are presented in Table 4 and the calibration curve is shown in Fig. 5.

LOD and LOQ

The LOD and LOQ identify the smallest concentrations that can be accurately and precisely determined using a specific method, with lower values indicating greater sensitivity. Both values were calculated

Table 2: Optimization results for flow rate selection

Flow rate	Area (mV/s)	Retention time (min)	Tailing factor (Tf)	HETP	Number of theoretical plates
0.8	103 629	2.151	1.127	51.377	2920
1.0	82 545	1.727	1.105	56.818	2640
1.2	100 705	1.435	0.739	74.85	2004

Table 3: System suitability test result

Area (mV/s)	Retention time (min)	Tailing factor (Tf)	HETP	Number of theoretical plates (n)	Standard deviation	Coefficient of variation (%)
107 559	2.145	1.304	47.515	3157		
110 499	2.143	1.351	47.754	3141	1826.222	1.667269601
109 938	2.150	1.308	47.505	3158		
110 752	2.144	1.296	48.747	3077		
106 990	2.144	1.299	47.972	3127		
111 464	2.148	1.45	47.515	2930		

Table 4: Calibration curve data, LOD, and LOQ of tranexamic acid

Concentration (mg/mL)	Area (mV/s)	S (y/x) ^{2}	S (y/x)	LOD (mg/mL)	LOQ (mg/mL)
150	56 257	30 074 678	5484.038	30.22509	100.8503
250	123 447				
300	141 759				
500	250 373				
600	301 124				
700	363 272				
n=6	Σ=12 029 8712				

LOD: Limit of detection, LOQ: Limit of quantification

Table 5: Data on the accuracy and precision of tranexamic acid content analysis in cream preparations

Concentration (µg/mL)	Area (µV/s)	Calculated concentration (µg/mL)	SD (%)	CV (%)	UPK (%)	Average (%)
239.8	112 720	245.0568245	1.74	1.72	102.19	101.175
	108 762	237.7781456			99.15	
	112 700	245.0200449			102.17	
300.1	141 027	297.112803	1.31	1.31	99.00	100.44
	142 770	300.3181434			100.07	
	143 386	301.4509544			100.45	
	146 719	307.5802714			102.49	
	144 873	304.1855162			101.36	
	141 437	297.8667844			99.00	
360.2	173 778	357.3412042	1.02	1.02	99.20	99.789
	173 747	357.2841958			99.19	
	177 235	363.6985546			100.97	

SD: Standard deviation, CV: Coefficient of variation

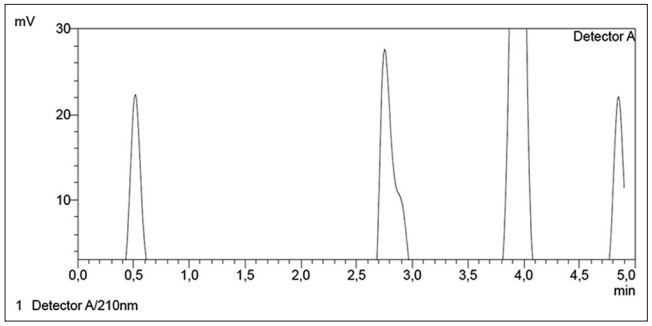

Fig. 3: Chromatogram of the cream placebo solution after extraction

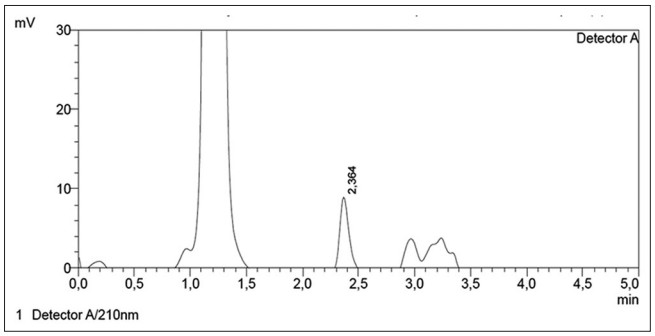

Fig. 4: Chromatogram of a sample solution of a commercially available whitening cream

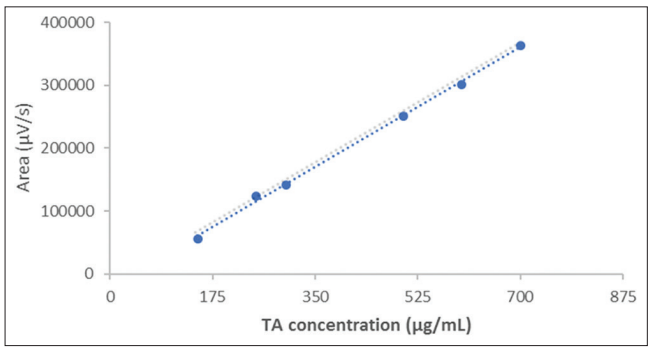

Fig. 5: Calibration curve of a standard solution of tranexamic acid

Table 6: Determination of TA levels in a commercially available bleach cream sample

Sample	Sample weighed (mg)	Area (µV/s)	Concentration (%)	Average (%)
X	150	61 851	1.00	
		63 785	1.03	1.013
		62 098	1.01	

TA: Tranexamic acid

statistically using a linear regression line from the calibration curve. The LOD for TA solution was 30.225 µg/mL, whereas the LOD was 100.85 µg/mL. The results of these analyses are presented in Table 4.

Accuracy and precision

Based on the results of the analysis, the average % recovery of TA at concentrations of 80, 100, and 120% was 101.175, 100.44, and 99.789%, respectively, which all met the criterion of 98–102%. Furthermore, the CVs of TA at these concentrations were 1.727, 1.316, and 1.023%, respectively. These data also met the criteria for accuracy and precision, indicating that this method is suitable for analyzing TA content. The results for recovery and CV obtained in the accuracy test illustrated that the extraction method optimally separated the analyte from the mixture. The results of this analysis are shown in Table 5.

Determination of TA levels in whitening cream samples

The determination of a commercially available whitening cream sample revealed that its TA content was 1.02%. This level does not exceed the limit in cosmetics of 1.5–2% [2]. From the results of the analysis, it can be concluded that the developed method can be used to analyze TA content in whitening creams. The results of the level determination data are presented in Table 6 and Fig. 4.

CONCLUSION

The optimal conditions for analyzing TA content in whitening cream preparations using reverse-phase HPLC were as follows: Water solvent, a C18 SunFire column (4.6 mm inner diameter size, 5 µm particle size, and 250 mm column length), UV-Vis detector, mobile phase consisting of acetonitrile: double-distilled water: phosphate buffer (64:34:2 v/v/v), wavelength of 210 nm, and flow rate of 0.8 mL/min. The injection volume was 20.0 µL. The retention time of the compound peak was in the 2nd min.

The analytical method fulfills all of the criteria for a validation method, including linearity, selectivity, precision, and accuracy, and thus the developed method was declared valid. The method is applicable for analyzing whitening cream samples, as the method identified that the TA content in a commercially available sample was 1.02%.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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