Short Communication

Facile and Accurate Analysis of Sodium Dehydroacetate in Cosmetic Powders After Extraction by Na₂CO₃-Methanol-H₂O and Derivatization with 4-Nitrophenylhydrazine·HCl

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Abstract

Sodium dehydroacetate (SDA) was extracted from cosmetic powders using a Na₂CO₃-methanol-H₂O system. After the extract was neutralized, the resulting dehydroacetic acid (DAA) reacted with 4-nitrophenylhydrazine·HCl (4-NPH·HCl) to give the corresponding hydrazone, which was determined by an HPLC-VIS (400 nm) system. Using 4-NPH·HCl in great excess was suitable for detecting the presence of DAA, which almost reacted with the hydrazine to yield the corresponding hydrazone. As a result, it was determined that contaminants in the cosmetic powder did not interfere with analysis of the hydrazone. Importantly, the molar absorptivity of the hydrazone (1.29×10^4 at 400 nm) is five times that of DAA (2.57×10^3 at 230 nm). Accordingly, detection of the hydrazone at 400 nm in a new analytical method might have fivefold higher sensitivity compared with that of DAA at 230 nm in the standard method.

Keywords: Sodium dehydroacetate; Dehydroacetic acid; Antiseptic; Cosmetic powder; 4-Nitrophenylhydrazine·HCl

1. Introduction

Sodium dehydroacetate (SDA) and dehydroacetic acid (DAA), an antiseptic, have been widely utilized in foods [1] such as cheese, butter and margarine and in cosmetics [2] such as face powder and lotion to prevent the spread of bacteria, mould, and other microorganisms. Standards have been officially set for the use of SDA and DAA in foods [3] and cosmetics [4], and its content must be determined according to the prescribed methods involving steam-distillation, followed by extraction of the distillate with diethyl ether and detection by absorption spectroscopy or high-performance liquid chromatography (HPLC) [5]. Among the frequently reported procedures for analyzing DAA in various types of samples [6–12], steam-distillation for the separation of DAA in samples has been widely adopted [6–8], followed by determination by other methods, including HPLC-UV (ultraviolet). Assessing the photoreactivities of additives in cosmetics has attracted much interest following the Hakuhan incident involving

rhododendrol manufactured by Kanebo Co. Ltd [13]. Accurately determining the amount of SDA as one of additives in cosmetics is essential, because SDA induces photoreactions including H-abstraction and epoxidation in our preliminary experiments. In the present study, we focused on the content of SDA in cosmetic powders. However, we were unsuccessful because determining the content of SDA in cosmetic powders is complicated by the possible presence of other compounds used as antiseptics, including *p*-hydroxybenzoic acid, benzoic acid, phenoxyethanol, hinokitiol, sorbic acid and SDA. The obstacles were based on two factors. First, SDA in cosmetic powder could not be obtained as DAA in sufficient quantities through steam-distillation under acidic condition using the standard method [5]. Second, the DAA partially separated through steam-distillation was directly analysed using HPLC-UV, which lacks the necessary selectivity because many compounds obtained from steam-distillation have similar absorbance profiles in the

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UV region.

To solve the first problem, we used a Na_2CO_3 -methanol- H_2O extraction system instead of steam-distillation. To solve the second problem, we converted the SDA obtained from the system to DAA, which reacted with 4-NPH·HCl (4-nitrophenylhydrazine·HCl) to produce a high yield of the corresponding hydrazone. Simultaneously the hydrazone absorbs in the VIS region at 400 nm without overlapping the UV absorption region based on the presence of the additive like sorbic acid. Such characteristic results in the selectivity on the analysis of SDA. 4-NPH·HCl [14] was selected from many hydrazine derivatives because of its high solubility in methanol which also acts as a part of solvent to dissolve DAA in the reaction.

It is well known that 2,4-dinitrophenylhydrazine reacts with carbonyl compounds to produce the corresponding hydrazones as crystalline solids, which are used for qualitative analysis of the carbonyl compounds. As this hydrazine dissolves slightly in acidic aqueous solution, and the corresponding hydrazones also dissolve slightly in organic solvents, 2,4-dinitrophenylhydrazine could not be selected. By contrast, it was found that 4-NPH·HCl dissolves thoroughly in methanol, which favourably affects the hydrazone yield obtained from the reaction of DAA with 4-NPH·HCl. The hydrazone dissolves in ethyl acetate, so it was used as extraction solvent for the reaction mixture.

In this study, we have reported a new analytical method for SDA in cosmetic powders including the extraction of SDA from cosmetic powders with Na_2CO_3 -methanol- H_2O system, the derivatization of DAA originating from the SDA by 4-NPH·HCl and the determination of the resulting hydrazone by HPLC-VIS, as shown in Chart 1.

Chart 1. A new analytical method for SDA in cosmetic powders using an HPLC-VIS system at 400 nm.

2. Experimental

2.1. Apparatus

Melting points were measured on a Yamato (Tokyo, Japan) melting point apparatus using a capillary tube and were not corrected. ^1H -NMR and MS spectra were taken on Varian (Palo Alto, CA, USA) 400-MR and JEOL (Tokyo, Japan) JMS-700 mass spectrometers, respectively. Elemental analysis was performed with a Yanako (Kyoto, Japan) CHN MT-6 analyzer. UV-VIS spectra were measured using a Hitachi (Tokyo, Japan) U-2000 spectrometer. A type 2410 centrifuge (KUBOTA, Tokyo, Japan) was used for separation between extraction solvent and cosmetic powder, and a type SB-9 water bath (Eyela, Tokyo, Japan) was used to control the reaction temperature. HPLC-VIS analysis was carried out at room temperature with a GL Sciences (Tokyo, Japan) PU 614 equipped with a UV-VIS detector adjusted to 400 nm and a 20 μL sample loop. A stainless steel column (4.6 mm i.d. $\times$ 250 mm) packed with Inertsil ODS-3 (5 μm , GL Sciences) was used. A mixed solution of acetonitrile and H_2O (7/3, v/v) was employed as the mobile phase at a flow rate of 1.0 mL min^{-1} .

2.2. Chemicals

Impress Loose Powder and CyberWhite Brilliant Perfection were purchased from Kanebo (Tokyo, Japan) and Estee Lauder (NY City, NY, USA), respectively. Pure nitrogen (99.999 %) was supplied by Taiyo-Nissan Kogyo (Tokyo, Japan). 4-NPH·HCl, DAA and SDA- H_2O were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), from Wako Pure Chemicals Industries (Osaka, Japan) and from Wako Pure Chemicals Industries, respectively, which were recrystallized from methanol, methanol and a mixture of methanol and benzene, respectively, before use. Other chemicals were supplied by Wako Pure Chemicals Industries.

DAA reacted with 4-NPH·HCl in a mixture of methanol and H_2O to yield the corresponding hydrazone, which was recrystallized from methanol to produce the hydrazone as yellow needles (yield, 56%), mp 227–228°C with decomposition. VIS λ_{max} ($\text{CH}_3\text{COOC}_2\text{H}_5$) nm (log ϵ): 400 (4.11). ^1H -NMR (400 MHz, acetonitrile- d_3 and $\text{DMSO}-\text{d}_6$ (3/1, v/v) δ : 2.18 (s, 3H, CH_3), 2.59 (s, 3H, $\text{N}=\text{C}-\text{CH}_3$), 5.96 (s, 1H, olefinic), 7.03 (d, 2H, $J = 9.4$ Hz, aromatic), 8.16 (d, 2H, $J = 9.4$ Hz, aromatic), 9.89 (br, 1H, NH), 15.57 (br, 1H, enolic) [15,16]. MS (EI, m/z) (relative intensity): 303 (100), 286 (15), 261 (45), 246 (100). Anal. Calcd for $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_7$: C, 55.45; H, 4.32; N, 13.86%. Found: C, 55.16; H, 4.27; N, 13.73%.

2.3. Sample preparation

Prior to the analytical procedure, a standard solution of the synthesized 4-nitrophenylhydrazone ($0.25 \mu\text{mol mL}^{-1}$ in ethyl acetate), a standard solution of SDA ($2.0 \mu\text{mol mL}^{-1}$ in methanol and H_2O (1/1, v/v)) containing $50 \mu\text{mol}$ of Na_2CO_3 per mL, a solution of the extraction solvent (methanol and H_2O (1/1, v/v)) containing $50 \mu\text{mol}$ of Na_2CO_3 per mL and a 4-NPH·HCl solution ($100 \mu\text{mol mL}^{-1}$ in methanol) were prepared. The sample solution was prepared as follows. Cosmetic powder (200 mg) was placed into a 10 mL tube fitted with a screw cap, followed by the addition of 5 mL of extraction solvent. The tube was vigorously shaken for 1 min and then left to rest for 5 min. After repeating this procedure twice, the tube was centrifuged for 15 min at 4×10^3 rpm, and the resulting solution was filtered through a $0.45 \mu\text{m}$ membrane filter. The analytical procedure was performed as follows. First, 0.5 mL of the standard solution of SDA and 0.5 mL of the sample solution were placed into separate 10 mL tubes with screw caps. Next, $50 \mu\text{L}$ of 1 mol L^{-1} hydrochloric acid was added to each tube, followed by mixing, and 0.5 mL of a 4-NPH·HCl solution was added to the solution, followed by mixing. The mixture was then allowed to stand for 45 min at 60°C . After the reaction mixture was cooled in ice-cold water for 30 min, 4 mL of H_2O and 4 mL of ethyl acetate were added to the mixture, followed by vigorous shaking for 1 min. The resulting ethyl acetate solution was analyzed by an HPLC-VIS system at 400 nm.

By using final concentrations of 0.4, 0.8, 1.2 and $1.6 \mu\text{mol mL}^{-1}$ SDA, the linear calibration curve ($r = 0.998$) was obtained. Simultaneously the detection limit was found to be 6 nmol mL^{-1} based on the definition in the Japanese Pharmacopoeia [17]. Additionally, the reproducibility was evaluated based on the standard deviation (S.D.).

3. Results and discussion

3.1. Reversed-phase HPLC separation of NPH derivatized DAA in the cosmetic powder

Figure 1 shows typical HPLC-VIS chromatograms obtained from a standard solution of the synthesized hydrazone, a standard solution of SDA and one of the sample solutions. In Fig. 1A, one peak corresponding to the synthesized hydrazone appeared at a retention time of 7.3 min. In Fig. 1B and Fig. 1C, an additional peak corresponding to the excess 4-NPH·HCl appeared at 3.9 min. These two peaks interfered little with each other. Contaminants in the cosmetic powder may have resulted in the small peak appearing in Fig. 1C, but this peak did not interfere with the analysis of SDA.

Thus, the HPLC-VIS chromatograms obtained by the new analytical method clearly distinguished 4-NPH·HCl,

Fig. 1. Typical chromatograms obtained from (A) standard solution of synthesized hydrazone, (B) standard solution of SDA and (C) one of the sample solutions. Peaks: 1, 4-NPH·HCl; 2, hydrazone. HPLC-VIS conditions are described in the text.

the hydrazone and the contaminants in the cosmetic powder.

3.2. Yield of hydrazone obtained by reaction of DAA derived from SDA with 4-NPH·HCl

After the standard solution of SDA was neutralized, 4-NPH·HCl solution was added, and the mixture stood for 30, 40 and 50 min at 60°C , respectively. The ethyl acetate solution of the hydrazone extracted from the reaction mixture was compared to the standard solution of synthesized hydrazone using an HPLC-VIS system. The yield of the hydrazone reached a maximum after approximately 40 min of reaction time. At 60°C , the yield was $89 \pm 2.2\%$ at 30 min, $95 \pm 1.4\%$ at 40 min and $94 \pm 1.6\%$ at 50 min. By contrast, the yield at 55°C was not satisfactory. Additionally, measurements of the hydrazone yield were carried out in 4-NPH·HCl solutions at concentrations of 30, 50 and $100 \mu\text{mol mL}^{-1}$. The $100 \mu\text{mol mL}^{-1}$ concentration led to a good yield, because the reaction condition leading to the hydrazone might require a higher concentration of 4-NPH·HCl to encounter DAA and a weak acid of 4-NPH·HCl as a catalyst [18] for dehydration. Considering the studied conditions, a 60°C reaction temperature, 45 min reaction time and $100 \mu\text{mol mL}^{-1}$ 4-NPH·HCl concentration were used to obtain the experimental data. Following the conversion of SDA to DAA, DAA reacted with 4-NPH·HCl to produce a satisfactory yield of the corresponding hydrazone.

3.3. Recovery of SDA from cosmetic powder

The recovery of SDA separated by steam-distillation under acidic condition was 25–83%, which was not

satisfactory. Therefore, we attempted to separate it directly by extraction with solvents such as benzene, ethyl acetate and diethyl ether under acidic conditions using HCl, H₂SO₄, tartaric acid and citric acid, but the attempt performed here was unsuccessful. Considering these results, it was suggested that SDA could not be liberated as DAA from cosmetic powders. Therefore, the separation of SDA was investigated using Na₂CO₃ as alkali agent in order to extract SDA, for which the agent was found to be effective. We therefore dissolved Na₂CO₃ in methanol and H₂O, and the resulting solution was employed as the extraction solvent of SDA in cosmetic powders.

It is known that cosmetic powders are manufactured by mixing with SDA owing to its solubility in H₂O. The following procedure was used to recover SDA from the cosmetic powders. Powder (200 mg) was placed into a 10 mL tube with a screw cap, followed by the addition of 200 µL of the SDA methanol solution (50 µmol mL⁻¹) and removal of methanol vapour in the tube under 1 L min⁻¹ of N₂ for 15 min. Five millilitres of the extraction solvent was added to the tube, which was then subjected to the sample preparation and analytical procedures. The results were shown in Table 1, in which the recovery was acceptable for the analysis of SDA in cosmetic powders.

Table 1. Recovery of sodium dehydroacetate (SDA) after its addition to cosmetic powder.

Sample	Recovery (%)
Impress Loose Powder	103 ± 3.0
CyberWhite Brilliant Perfection	98 ± 1.9

Data represented as the mean ± S.D. of three experiments.

3.4. Determination of SDA in cosmetic powder

Using the analytical procedure described above, SDA in two cosmetic powders, Impress Loose Powder and CyberWhite Brilliant Perfection, was examined three times. The amount of SDA in these cosmetic powders on the market was not disclosed by the manufacture. However, the presence of SDA in cosmetic powders was confirmed. The results were shown in Table 2. According to the standard for SDA in cosmetics set by the Ministry of Health and Welfare at 500 mg in 100 g of the cosmetic [4], the amounts determined in these samples were found to be within the standard for use in Japan. Accordingly, it is important to know the photoreactivity of SDA because the compound might cause skin injury.

4. Conclusion

In this paper, we recognized three facts of primary importance. First, the extraction of SDA in cosmetic powders was facilitated by Na₂CO₃-methanol-H₂O system.

Table 2. Amount of sodium dehydroacetate (SDA) in cosmetic powder.

Sample	Amount (mg/100g)
Impress Loose Powder	55 ± 1.7
CyberWhite Brilliant Perfection	124 ± 7.0

Data represented as the mean ± S.D. of three experiments.

Second, using 4-NPH·HCl in great excess was suitable for detecting the presence of DAA originating from the SDA, which almost reacted with the hydrazine to yield the corresponding hydrazone. Third, contaminants in cosmetic powders did not interfere with the analysis of the hydrazone by an HPLC-VIS system. Based on the molar absorptivity, detection sensitivity of the hydrazone at 400 nm in the new analytical method might be fivefold higher than that of DAA at 230 nm in the previous method. Accordingly, the new analytical method for SDA in cosmetic powders was found to be facile and accurate compared with the standard method by using steam-distillation for the separation of DAA and the following HPLC-UV analysis without the derivatization of DAA.

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