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Simultaneous analysis of dehydroacetic acid, benzoic acid, sorbic acid and salicylic acid in cosmetic products by solid-phase extraction and high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic (HPLC) method for simultaneous determination of dehydroacetic acid (DHA), benzoic acid (BA), sorbic acid (SOA) and salicylic acid (SA) was developed for application to cosmetic products. Isocratic reversed-phase HPLC was employed for quantitative analysis using tetra-*n*-butylammonium (TBA) hydroxide as an ion-pair reagent. Cosmetic samples were purified by solid-phase extraction using Bond–Elut SI cartridges. Four acidic preservatives were eluted with methanol from cartridges. The HPLC assay was carried out using TSK gel ODS-80TM column (5 μm, 150 × 4.6 mm I.D.). The mobile phase consisted of a mixture of water and methanol (65:35, v/v) containing 2.5 mM TBA hydroxide adjusted with phosphoric acid to pH 7.0. The calibration curves of these preservatives showed good linearity with UV detection (235 nm). The correlation coefficients were better than 0.999 in all cases. The lower limits of detection (defined as a signal-to-noise ratio of about 3) were approximately 2.5 ng for DHA, 4.0 ng for BA, 2.0 ng for SOA and 5.5 ng for SA. The procedure described here is simple, selective and is suitable for quality control of finished cosmetic products.

Keywords: Dehydroacetic acid; Benzoic acid; Sorbic acid; Salicylic acid; Cosmetics; Solid-phase extraction

1. Introduction

Single preservatives, but more often combinations of preservatives, are commonly used in cosmetic products to prevent alteration and degradation of the product formulations. However, these preservatives may be harmful to consumers due to their tendency to induce allergic contact dermatitis. Dehydroacetic acid (DHA), benzoic acid (BA), sorbic acid (SOA), salicylic acid (SA) and their sodium salts have been widely used as antimicrobial agents in pharmaceuticals

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and cosmetics because of their broad antimicrobial spectrum with good stability and non-volatility [1]. Hence, the simultaneous determination of these preservatives in commercial cosmetic products is particularly important both for quality assurance and consumer safety.

Diethyl ether extraction as a sample clean-up procedure for oil-rich cosmetics (milk lotion and cream) is frequently used for determination of preservatives by high-performance liquid chromatography (HPLC) [2–4]. However, the liquid–liquid extraction method is laborious, time-consuming and insufficient for removal of interfering substances. To remove substances that might interfere with determination, we established a sample clean-up procedure using solid-phase extraction in normal phase mode, using Bond–Elut SI cartridges for simultaneous analysis of these acidic preservatives in cosmetics by HPLC with UV detection.

Many HPLC methods have been developed for the determination of acidic preservatives in cosmetics [4–8]. However, there have been no reports concerning the simultaneous determination of DHA, BA, SOA and SA by solid-phase extraction and ion-pair isocratic HPLC.

Here, we describe a procedure for simultaneous determination of four acidic preservatives in cosmetics using reversed-phase ion-pair isocratic HPLC. Solid-phase extraction on Bond–Elut SI cartridges was applied to remove endogeneous interference in the analysis by UV detection. This procedure was used to determine DHA, BA, SOA and SA in commercial cosmetics.

2. Experimental

2.1. Chemicals and reagents

BA, SOA, SA, tetra-*n*-butylammonium (TBA) hydroxide solution (0.5 M, for HPLC) and HPLC-grade methanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). DHA was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade.

2.2. Apparatus

A Shimadzu (Kyoto) HPLC system consisting of the following components was used, a model LC-6A pump; a model CTO-10A column oven; a model SPD-6A UV detector; a model C-R6A integrator/recorder; and a model SIL-10AD auto injector fitted with a 50 μl injection loop. Column temperature was 40 °C and chromatographic separation was carried out on TSK gel ODS-80TM column (5 μm, 150 × 4.6 mm I.D., TOSOH Co., Tokyo, Japan). An ultrasonic bath (model B-42H, Branson Co., Shelton, CT, USA) was used to dissolve the samples in methanol.

For sample preparation, Bond–Elut SI cartridges (3 ml capacity containing 500 mg of sorbent; Varian, Harbor City, CA, USA) preconditioned with 4 ml of *n*-hexane:ethyl acetate (4:1, v/v) were utilized. The cartridges were prevented from drying. A vacuum manifold capable of holding 12 sample cartridges (GL Sciences, Tokyo, Japan) was used for simultaneous sample extraction.

2.3. Chromatographic conditions

Chromatography was performed under isocratic conditions, at a flow-rate of 1.0 ml/min. The mobile phase consisted of a mixture of water and methanol (65:35, v/v) containing 2.5 mM TBA hydroxide adjusted with phosphoric acid to pH 7.0. The column effluent was monitored at 235 nm using a detector range of 0.04 absorbance unit of full scale (aufs). The chart speed was 1 mm/min. An aliquot of sample solution (10 μl) was injected onto the analytical column with an auto HPLC injector.

2.4. Calibration curves of standard solutions

Standard stock solutions of DHA (1.0 mg/ml), BA (1.0 mg/ml), SOA (1.0 mg/ml) and SA (1.5 mg/ml) were prepared by dissolving appropriate amounts of respective compounds in methanol at room temperature. All stock solutions were stored at −20 °C in a freezer.

Aliquots (5 ml) of standard stock solutions were pipetted into a same 50 ml volumetric flask,

and then diluted to volume with methanol. Then, aliquots (0.5, 2, 4, 6 and 10 ml of this DHA, BA, SOA and SA diluted standard solution) were pipetted into 50 ml volumetric flasks and diluted to volume with methanol:water (1:1, v/v). Mixed standards containing 1, 4, 8, 12 and 20 μg/ml of DHA, BA and SOA, and 1.5, 6, 12, 18 and 30 μg/ml of SA were prepared.

An aliquot (10 μl) of each standard was injected. All measurements were performed in duplicate for each concentration on 6 different days. The peak areas were measured and those of analytes (y) were plotted against the respective concentration (μg/ml) of DHA, BA, SOA and SA (x). Least square linear regression analysis was used to determine the slope, y-intercept and the correlation coefficients of the standard plots.

2.5. Sample preparation

The cosmetic product (1 g) was accurately weighed into a 25 ml NASU flask and concentrated in a boiling water bath for 30 min. The concentrate was dissolved in 5 ml of ethyl acetate by sonication. After dilution with n-hexane to 25 ml, a portion (1 ml) of the resulting sample was applied at a flow-rate of ca. 1 ml/min to a Bond–Elut SI cartridge. The extraction column was washed with 4 ml of n-hexane:ethyl acetate (4:1, v/v), lightly dried by vacuum and eluted with 4 ml of methanol. The final fraction was evaporated to dryness using a water bath (40 °C). The residue was dissolved in 5 ml of methanol:water (1:1, v/v) and an aliquot (10 μl) was analyzed by HPLC.

2.6. Assay validation

Milk lotion and cream (oil-in-water emulsion) test samples were prepared in the laboratory by spiking with diluted DHA, BA, SOA and SA standard solutions to the formulation components (milk lotion excipients were 1,3-butylene glycol, liquid petrolatum, 2-octyldodecanol, behenil alcohol, sorbitane monostearate, polyethyleneglycol monostearate, ethyl parahydroxybenzoate, water and cream excipients were glycerin, stearic acid, glyceryl tri(2-ethylhexanoate), cetyl palmitate, squalane, sorbitane monostearate, jojoba oil, 2-octyldodecanol, water). The reproducibility was verified by spiking the formulations at three concentration levels of 0.25, 1.0 and 2.0 mg/g for DHA, BA and SOA, and 0.38, 1.5 and 3.0 mg/g for SA. The recovery (%) was also calculated.

The accuracy and precision of the method were evaluated by analyzing samples from the same milk lotion and cream formulations on the same day (within-day, $n = 6$) and on different days (between-day, $n = 6$).

3. Results and discussion

3.1. Chromatography

In the analyses of these compounds, BA, SOA and SA were found to be weakly retained on the reversed-phase sorbents with a mixture of water and methanol (65:35, v/v). They showed reduced resolution, and increased interference from unretained matrix compounds, which hampered accurate determination. The addition of alkylammonium cations to the eluent is particularly useful for the separation and detection of several sulfonated azodyes [9], steroid sulfate [10] and anthranilic acid derivatives [11], because it provides appropriate capacity factors for complexes formed with the counter ion. Therefore, to enhance the retention of three preservatives on reversed-phase HPLC, the ion-pair reagent TBA hydroxide was used as an additive.

When the effects of mobile phase pH on peak shapes were examined, DHA displayed broad and tailing peak with acidic mobile phase [12]. A pH value of 7.0 in the mobile phase was selected to define well the DHA peak and to ensure ionization of preservatives, and also ion-pair formation with the TBA without the eluents damaging the silica packing materials.

The effects of TBA concentration were examined under various conditions in which the four preservatives were eluted within 15 min. As shown in Fig. 1, the capacity factor, k', of the analyte was enhanced by increasing the TBA concentration from 0.5 to 5 mM. A concentration of 2.5 mM was selected due to optimum peak shape, resolution and elution of the compounds within

13 min. Utilizing these chromatographic conditions, the capacity factors of DHA, BA, SOA and SA were 3.1, 4.1, 5.0 and 6.8, respectively.

3.2. Solid-phase extraction

Solid-phase extraction techniques are useful in the analysis of cosmetic products [13–17]. In general, the oil consisted of hydrocarbons, fatty alcohols, fatty acids, wax and glycerides is present to a maximum of about 25% in milk lotions and about 70% in creams [18]. Although C18 silica cartridges have been employed for the extraction of four preservatives from water samples [19,20], a problem remaining unresolved is the employment of the same pre-treatment procedure for all samples, which is an important factor. We examined to purify four acidic preservatives using Bond–Elut SAX (silica bonded to quaternary ammonium groups, strong anion exchange) and PSA (silica bonded to primary/secondary ammonium groups, weak anion exchange) cartridges. However, SAX and PSA cartridges were found to inefficiently retain DHA and SOA in various solvents (i.e. water, tetrahydrofuran). Consequently, we selected Bond–Elut SI cartridges pre-packed with polar silica sorbent for oil-rich cosmetics since ethyl acetate was used for the effective dissolution of concentrated cosmetic matrices. To test the suitability of the four preservatives in organic solvents and of the eluting solvent, extraction was first tested without cosmetic matrix, so that these

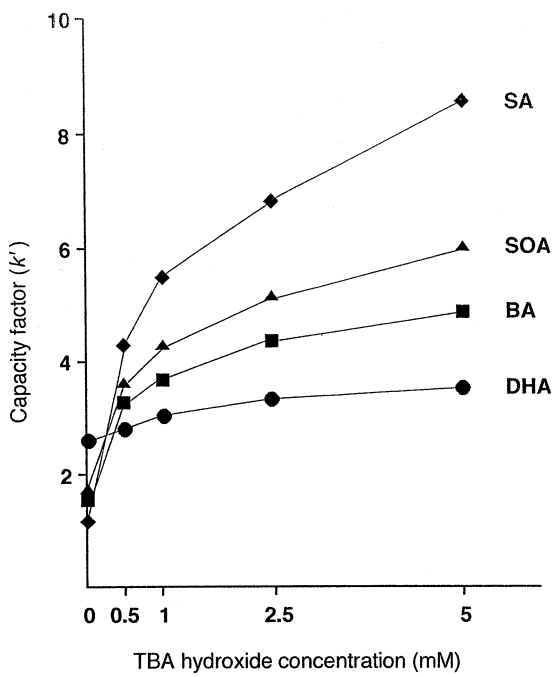

Fig. 1. Effects of TBA hydroxide concentration on the capacity factor, k', of DHA, BA, SOA and SA. The mobile phase also contained water–methanol (65:35, v/v) and was adjusted with phosphoric acid to pH 7.0. The other chromatographic conditions are described in the text.

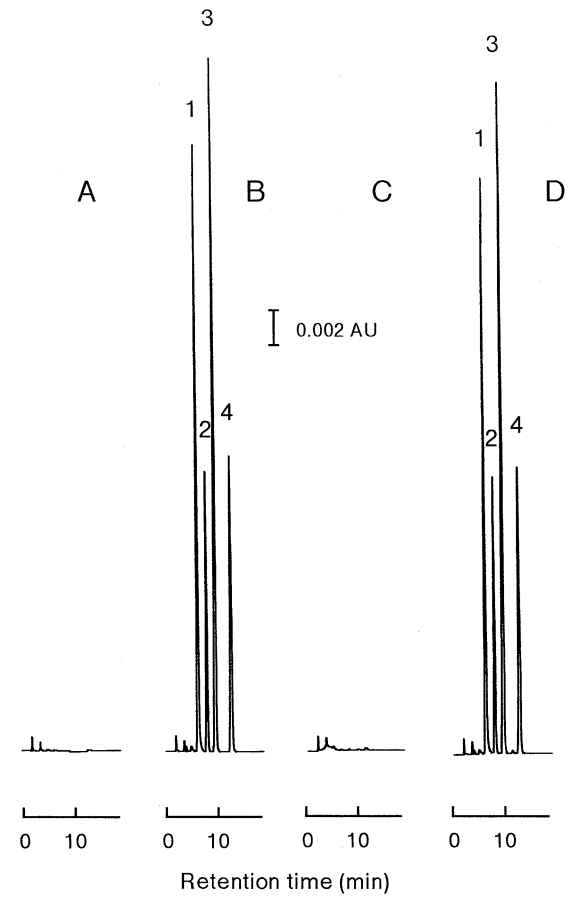

Fig. 2. Chromatograms of (A) a milk lotion placebo extract, (B) the same lotion spiked with 0.8 mg/g for DHA, BA and SOA, and 1.2 mg/g for SA, (C) a cream placebo extract, (D) the same cream spiked with 0.8 mg/g for DHA, BA and SOA, and 1.2 mg/g for SA as the final concentration. Chromatographic conditions were described in the text. Peaks, 1, DHA; 2, BA; 3, SOA; 4, SA.

Table 1
Within-day precision for acidic preservatives added to experimental formulations ($n = 6$)

Sample	DHA			BA			SOA			SA		
	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)
Milk lotion	0.5	95.6 ± 4.1	4.3	0.5	99.0 ± 2.6	2.6	0.5	97.5 ± 2.2	2.3	0.38	98.6 ± 3.0	3.0
	1.0	98.1 ± 3.2	3.3	1.0	97.8 ± 2.5	2.6	1.0	99.0 ± 2.1	2.1	1.5	97.1 ± 2.1	2.1
	2.0	95.8 ± 2.9	3.0	2.0	97.5 ± 3.1	3.2	2.0	98.7 ± 2.8	2.8	3.0	96.8 ± 2.2	2.3
Cream	0.5	96.1 ± 3.5	3.6	0.5	97.6 ± 2.4	2.5	0.5	96.5 ± 3.0	3.1	0.38	97.6 ± 2.8	2.9
	1.0	97.0 ± 3.7	3.8	1.0	98.4 ± 3.0	3.0	1.0	97.9 ± 1.8	1.8	1.5	98.1 ± 2.7	2.8
	2.0	96.4 ± 3.9	4.0	2.0	97.2 ± 1.7	1.7	2.0	97.0 ± 1.8	1.9	3.0	96.9 ± 2.6	2.7

Table 2
Between-day precision for acidic preservatives added to experimental formulations ($n = 6$)

Sample	DHA			BA			SOA			SA		
	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)	Amount added (mg/g)	Recovery (%, mean ± S.D.)	R.S.D. (%)
Milk lotion	0.5	94.8 ± 3.9	4.1	0.5	97.3 ± 3.3	3.4	0.5	98.1 ± 3.2	3.3	0.38	98.3 ± 3.5	3.6
	1.0	96.1 ± 3.7	3.9	1.0	97.1 ± 2.5	2.6	1.0	97.6 ± 2.4	2.5	1.5	97.5 ± 2.4	2.5
	2.0	95.6 ± 3.6	3.8	2.0	98.2 ± 2.5	2.5	2.0	97.0 ± 3.1	3.2	3.0	95.6 ± 2.8	2.9
Cream	0.5	95.9 ± 3.2	3.3	0.5	98.5 ± 3.0	3.0	0.5	97.9 ± 3.3	3.4	0.38	97.0 ± 3.3	3.4
	1.0	96.4 ± 3.8	3.9	1.0	98.1 ± 2.7	2.8	1.0	98.9 ± 2.9	2.9	1.5	97.7 ± 3.5	3.6
	2.0	97.2 ± 4.0	4.1	2.0	97.4 ± 2.2	2.3	2.0	96.8 ± 2.0	2.1	3.0	97.2 ± 2.1	2.2

Table 3
Assay results for HPLC analysis of acidic preservatives in commercial cosmetic products

Sample	Preservative	Label claim (mg/g)	Found (mg/g)[a]	Percent of label	R.S.D. (%)
Milk lotion 1	BA	1.8	1.75	97.4	3.1
Milk lotion 2	DHA	0.8	0.77	96.3	3.8
Cream 1	DHA	1.0	0.95	95.1	3.4
Cream 2	BA	1.2	1.14	94.8	3.0
Cream 3	SOA	0.5	0.48	96.2	2.5
Shampoo	BA	2.8	2.75	98.3	2.3
	SA	1.0	0.96	96.0	2.9
Tooth paste	BA	2.7	2.62	97.0	3.1
Hair liquid	SA	1.8	1.76	97.8	2.6

[a] Each value is the mean of five determinations.

compounds could be efficiently retained on Bond–Elut SI cartridges.

After washing the extraction cartridges with *n*-hexane:ethyl acetate (4:1) to remove non-polar endogeneous components, quantitative desorption of four preservatives was obtained using 4 ml of methanol. Typical interference (i.e. paraben) was excluded by *n*-hexane:ethyl acetate (4:1) as the wash solvent. This resulted in improved sensitivity and recovery.

3.3. Accuracy and precision

Reproducibility was determined by recovery experiments. Cosmetic milk lotion and cream placebos were spiked at concentration levels of 0.25, 1.0 and 2.0 mg/g for DHA, BA and SOA, and 0.38, 1.5 and 3.0 mg/g for SA, respectively, and analyzed accordingly to the method. Recovery and relative standard deviation (R.S.D.) data are presented in Tables 1 and 2. The recoveries of each preservative was $>94\%$ at each concentration examined. Precision calculated as RSD was $<4.5\%$.

3.4. Selectivity

The selectivity of the procedure was evaluated by analyzing 50 different commercially available cosmetic preparations. The chromatograms clearly indicated that there was no interference from the formulation excipients. Fig. 2 shows the comparative HPLC traces of placebos (Fig. 2A and C) and spiked placebos (Fig. 2B and D) for

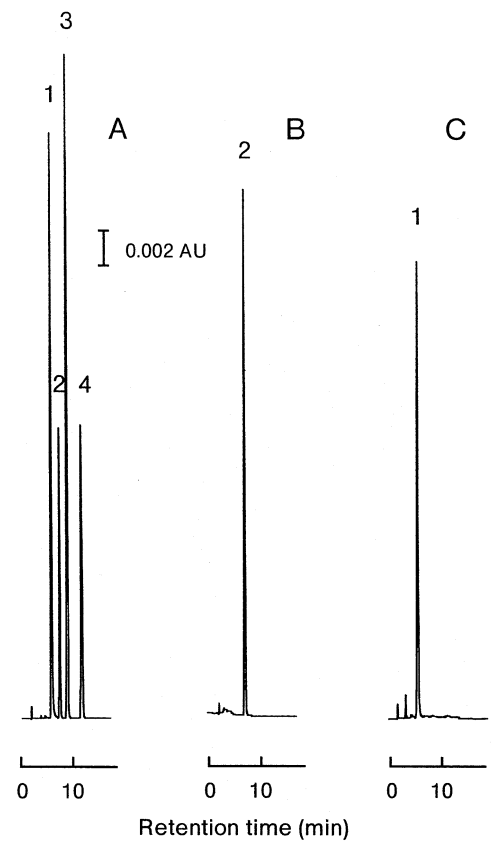

Fig. 3. Chromatograms of commercial cosmetic products, (A) standard solution containing 8 μg/ml for DHA, BA and SOA, and 12 μg/ml for SA; (B) milk lotion containing BA; (C) cream containing DHA. Chromatographic conditions were described in the text. Peaks, 1, DHA; 2, BA; 3, SOA; 4, SA.

the milk lotion and cream preparations. In contrast, direct injection of the samples onto the HPLC, after dissolution of the product in ethanol–water (1:1, v/v) and filtration, produced spurious peaks in the four preservative retention windows and late-eluting substances that interfered with subsequent analyses.

3.5. Linearity and quantification limit

Calibration curves ($n = 6$) were linear in the range of 1–20 μg/ml for DHA (slope, 1901.5 ± 14.8), 1–20 μg/ml for BA (slope, 850.3 ± 5.7), 0.5–10 μg/ml for SOA (slope, 2411.8 ± 42.6), and 1.5–30 μg/ml for SA (slope, 829.5 ± 4.9). The correlation coefficients were > 0.999 in all cases. The intercepts with the y-axis were not significantly different from the origin. The instrumental detection limits based on a signal-to-noise ratio of about three for standard solutions of DHA, BA, SOA and SA were 2.5, 4.0, 2.0 and 5.5 ng, respectively. The lower limits of quantification in cosmetics were 0.03 mg/g for DHA, 0.05 mg/g for BA, 0.02 mg/g for SOA and 0.07 mg/g for SA, using the procedure described above.

3.6. Application

Nine different commercially available cosmetic preparations, were assayed using the procedure developed in this study (Table 3). Typical chromatograms (for milk lotion one and cream one) are depicted in Fig. 3. The results obtained confirm the precision of the method and confirm the label claim on the products. The assay results indicate that the proposed analytical method is adequate for determination of DHA, BA, SOA and SA in commercial cosmetics.

4. Conclusions

An isocratic HPLC method using TBA hydroxide as an ion-pair reagent was established for the simultaneous analysis of DHA, BA, SOA and SA. For cosmetic products, the method could be used to remove endogeneous interference by solid-phase extraction on Bond–Elut SI cartridges as a sample clean-up procedure, thereby enabling efficient extraction and clean-up as well as being free of interference from excipients normally used in cosmetic formulations. Due to the minimal sample preparation, accuracy and precision, this method is acceptable for analyzing finished cosmetic products.

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