Determination of panthenol, cholecalciferol and tocopherol in cosmetic products by gas chromatography-mass spectrometry in SIM mode

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Abstract

A novel simple method to detect vitamins in cosmetic products by gas chromatography-mass spectrometry(GC-MS) has been developed. Three vitamins(panthenol, cholecalciferol and tocopherol) were used for this study. Vitamins were prepared by dissolving in tetrahydrofuran(THF), and silylated with bis-trimethylsilyltrifluoroacetamide-trichloromethylsilane(BSTFA). Silylated vitamins were separated on a fused-silica capillary column coated with DB-5. The identification of each vitamin was accomplished by retention time and mass spectrum library search with a computer, and the quantitation was made in the selected-ion monitoring(SIM) mode of GC-MS. SIM mode had given sensitivity to determine 50pg of panthenol, 285pg of cholecalciferol and 130pg of tocopherol. Linearity was maintained over the range 0.005-0.20% for each vitamin. Each cosmetic product(i.e. hair tonic and lotion) was found to contain amounts of the vitamins. This method was sensitive and gave 77.5-99.9% recovery of each vitamin from these cosmetic products. From these results, we concluded that silylation with BSTFA followed by GC-MS analysis allows the simple, convenient and exact determination of panthenol, cholecalciferol and tocopherol.

1. Introduction

Vitamins are bioactive materials having a variety of functions. Panthenol(vitamin B_5) is widely used for hair tonic and skin care products as moisturizer. Cholecalciferol(vitamin D_3) is important for the regulation of calcium and phosphorus metabolism. Tocopherol(vitamin E) is also incorporated as a widespread anti-oxidant in cosmetic products.

To detect the vitamins in cosmetic products, thin-layer chromatography(TLC)[1,2], high-performance liquid chromatography(HPLC)[3-5], and gas-liquid chromatography(GC)[6-8] have been used. Confirmation of vitamins by these methods has been made on the basis of retention time, and spectral characteristics using UV spectrophotometers or fluorometers.

Recently, a gas chromatography-mass spectrometry(GC-MS) method for the identification of vitamins was reported[9,10]. The GC-MS method in selected-ion monitoring(SIM) mode allowed the accurate determination and confirmation of vitamins among many ingredients of the cosmetic products. We propose a new simple method based on GC-MS for the detection of panthenol, cholecalciferol and tocopherol in cosmetic products: simple silylation using BSTFA increases the sensitivity and volatility, and eliminates the requirement for the complete removal of water from all solvents and samples.

2. Experimental

2.1. Reagents and materials

Benzylbenzoate, panthenol, cholecalciferol, tocopherol, tetrahydrofuran(THF) and *bis*-trimethylsilyltrifluoroacetamide-1% trichloromethylsilane(BSTFA) were purchased from Sigma(USA). All reagents were analytical grade and used without further purification.

2.2. Instruments and conditions

Analysis of vitamins was performed on a Model HP 5890 Series II gas chromatography(Hewlett-Packard, USA) interfaced with a Model HP 5971A mass selective detector and a Model HP 7673A automatic sampler. Electron impact(EI) mass spectra were recorded at an ionization potential of 70eV. The mass spectrometer was scanned from *m/z* 35 to 550 rate of 1.1 scan per second. Fused-silica capillary column(DB-5, J&W Scientific, USA), 30m x 0.25mm I.D., coated with DB-5 of 0.25μm film thickness was used.

Both the injection and the transfer line temperature were 280°C. The oven temperature was held at 150°C for 2min, then increased at a rate of 25°C/min to a final temperature of 280°C, and kept there for 10min. The carrier gas was helium at a column head pressure of 9 p.s.i.(1 p.s.i.=6894.76 Pa) with the split ratio of 20:1. Using an automatic sampler, 1ml-aliquots of the standard and sample solutions were analyzed under the operation conditions described above.

Quantitation was made based on peak area with GC-MS in SIM mode using benzylbenzoate as an internal standard.

2.3. Standard solutions

Standard stock solutions were prepared by dissolving the appropriate amount of vitamins in THF. A set of working standard solutions was made by diluting aliquots of the stock solutions with THF to 100ml in each volumetric flask containing 2ml internal standard solution. Then, each 200-μl aliquot of the mixed solutions was transferred to an automatic sampler vial and 200μl of BSTFA added. The concentration of each compound for the calibration curves was in the range of 0.01-5mg/ml.

2.4. Sample solutions

About 5g of cosmetic product was weighed into a 100-ml one-neck round flask, dissolved in about 5ml of THF by sonication and evaporated to dryness at 35°C under vacuum. Then the residue was dissolved in about 3ml of THF again, transferred into 10-ml volumetric flask, and pooled with three 2ml washings and 0.2ml internal standard solution to make 10ml. After filtration through a 0.45-μm membrane filter, each 200μl aliquot was transferred to an automatic sampler vial and then 200ml of BSTFA added.

2.5. Internal standard solutions

About 0.5g of benzylbenzoate was weighed into a 50-ml volumetric flask and diluted to the volume with THF.

3. Results and discussion

The total ion chromatogram(TIC) and selected-ion chromatogram of standard solution obtained with the proposed GC-MS method are shown in Fig.1 and 2. The electron impact(EI) mass spectra of the benzylbenzoate, panthenol, cholecalciferol and tocopherol are shown in Fig.3 through 6. Panthenol showed intense peaks at *m/z* 277 due to $[M\text{-}C_6H_{18}O_2Si_2]+$ ion fragment, and cholecalciferol and tocopherol showed strong ion peaks at *m/z* 325 and *m/z* 237 as base ions. The base and characteristic mass ion peaks of the vitamins are listed in Table 1.

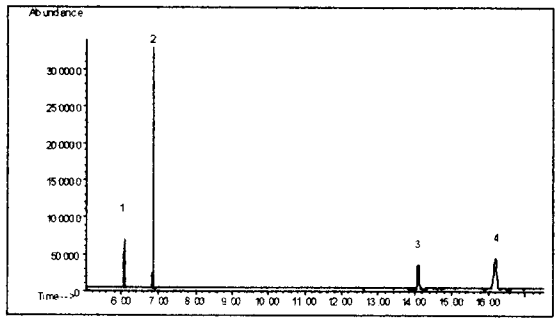

Fig. 1. Total ion chromatogram(TIC) of standard solution(1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

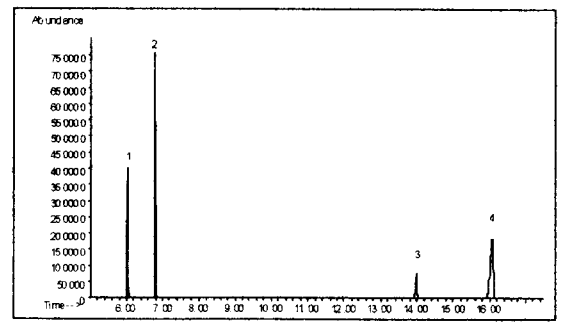

Fig. 2. Selected-ion chromatogram of solution(1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

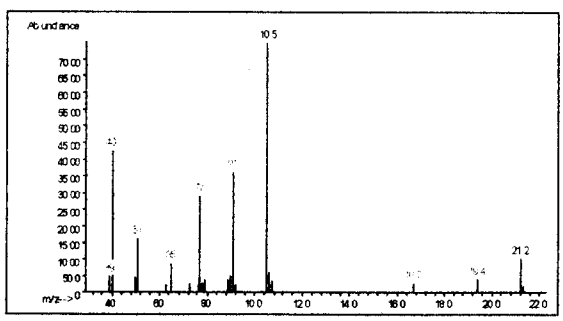

Fig. 3. EI mass spectrum of benzylbenzoate

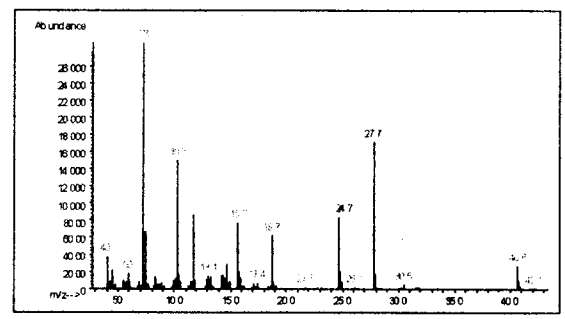

Fig. 4. EI mass spectrum of panthenol

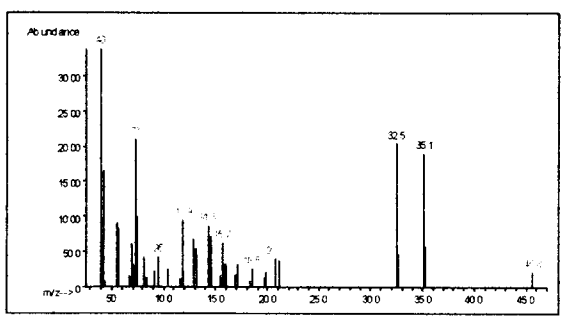

Fig. 5. EI mass spectrum of cholecalciferol

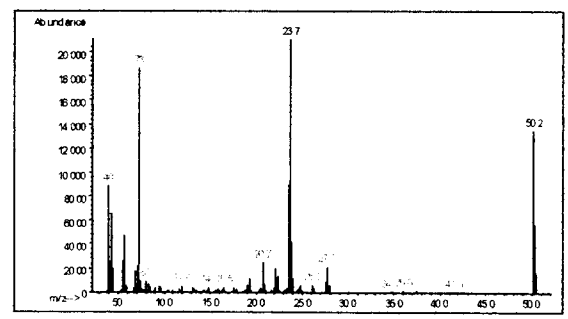

Fig. 6. EI mass spectrum of tocopherol

Table 1. The base and characteristic mass ion peaks of the vitamins

Peak No.	Vitamin	m/z	m/z
1	Benzylbenzoate(I.S.)	105 $[M-C_7H_7O]+$	212 [M]+
2	Panthenol	277 $[M-C_6H_{18}O_2Si_2]+$	247 $[M-C_8H_{24}O_2Si_2]+$
3	Cholecalciferol	325 $[M-C_6H_{14}Osi]+$	351 $[M-C_4H_{12}OSi]+$
4	Tocopherol	237 $[M-C_{19}H_{39}]+$	502 [M-H]+

I.S. : Internal standard

A calibration curve was constructed by plotting the peak areas against the concentration of the standards injected in GC-MS-SIM mode, and correlation coefficients, linear concentration ranges and detection limits are summarized in Table 2. Correlation coefficients were in the range of 0.999-1.000, and linear relationship was maintained over the range of 0.005-0.20%.

Table 2. The linear concentration ranges, correlation coefficients and detection limits of vitamins measured in GC-MS-SIM mode

Vitamin	Linear concentration range (%)	Correlation coefficient	Detection limit (pg)
Panthenol	0.01 - 0.20	0.99991	50
Cholecalciferol	0.01 - 0.10	0.99926	285
Tocopherol	0.005 - 0.05	0.99999	130

SIM mode had given good sensitivity to determine 50pg of panthenol, 285pg of cholecalciferol and 130pg of tocopherol further to 2-4ng(FID) of panthenol by A.R. Prosser *et al.*[7], 15pg of 1,25-dihydroxy cholecalciferol in the "high-tune" by R. Coldwell[11] and 170pg of tocopherol by D.W. Thomas *et al.*[12].

Recovery tests were carried out to evaluate the reproducibility and accuracy of the proposed method. We used a hair tonic for market without vitamins, and a skin care lotion made by formula shown in Table 3. Hair tonic and lotion were spiked with the amounts of agents reported in Table 4 and subjected to the described analytical procedure.

Table 3. Fomular of lotion

Ingredient	content (%)
Squalane	8.5
Polysorbate 60	1.0
Triethanolamine	0.2
Carbomer	0.2
Water	90.1

Table 4. Recoveries of vitamins from cosmetic samples

Vitamin	Amount added (%)	Recovery ± S.D.(%)	
		Hair tonic	Lotion
Panthenol	0.1	99.3 ± 0.6	99.9 ± 1.5
Cholecalciferol	0.2	77.5 ± 0.8	97.3 ± 1.9
Tocopherol	0.1	98.8 ± 2.1	96.7 ± 3.2

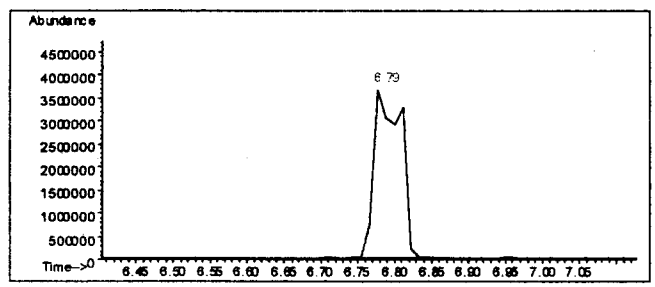

Fig. 7. TIC of the overlapped peak in hair tonic

In a Fig. 7, the peaks of the vitamins in practical GC analysis were overlapped with those of other ingredients in cosmetic products. In this case, the GC-MS information on the characteristic molecular and major ions helped to detect and determine the exact amounts of vitamins in cosmetic products.

Recovery with precision was observed, but cholecalciferol in a hair tonic was shown poor recovery of 77.5%. The reason for this is under investigation.

Total ion chromatogram(Fig. 8-9) and selected ion chromatogram(Fig. 10-11) of hair tonic and lotion showed that GC-MS-SIM mode excluded the unwanted peaks of ingredients in cosmetic products and determine the amounts of panthenol, cholecalciferol and tocopherol with accuracy. Therefore GC-MS-SIM mode could be used as a selective detector for panthenol, cholecalciferol and tocopherol.

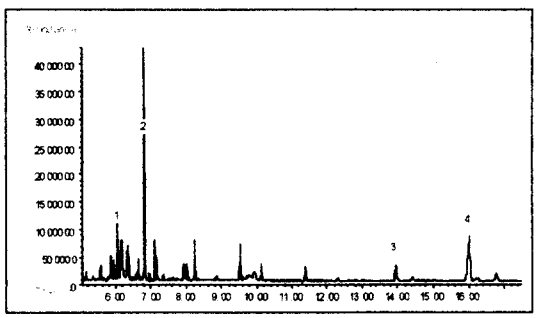

Fig. 8. TIC of hair tonic(1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

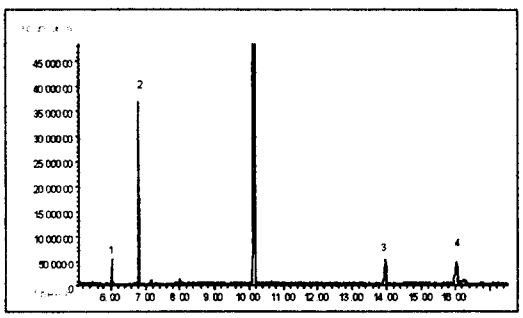

Fig. 9. TIC of lotion(1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

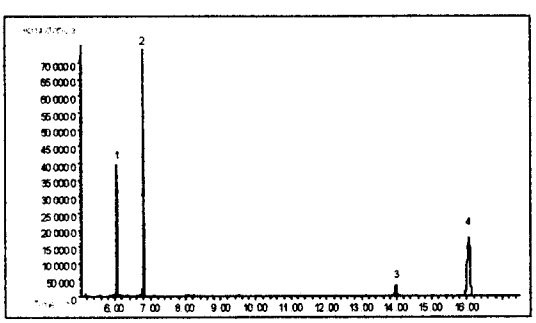

Fig.10. Selected-ion chromatogram of hair tonic (1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

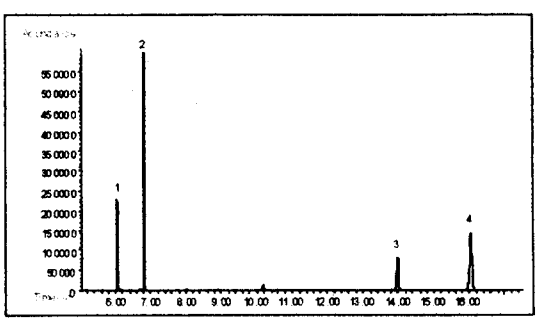

Fig. 11. Selected-ion chromatogram of lotion(1:Benzylbenzoate, 2:Panthenol, 3:Cholecalciferol, 4:Tocopherol)

4. Conclusion

A novel method, GC-MS following a simple, rapid silylation with BSTFA, was developed for the determination of panthenol, cholecalciferol and tocopherol in cosmetic products.

This method allowed a simultaneous, simple, rapid and accurate determination and confirmation of panthenol, cholecalciferol and tocopherol in cosmetic products containing many ingredients.

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