



Determination of benzophenones in a cosmetic matrix by supercritical fluid extraction and capillary electrophoresis

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Abstract

Benzophenones are common additives in commercial sunscreen products. These UV absorbers were extracted by supercritical fluid extraction (SFE), and then analyzed by capillary zone electrophoresis (CZE) and high-performance liquid chromatography. This study was applied to identify the contents of benzophenones in cosmetic matrix, and simultaneously, to investigate the behavior of migration in CZE. Adding non-ionic surfactant (Tween 20) to optimize the separation in the CZE operation improves resolution. In SFE, we chose carbon dioxide with 2.5% modifier of 10% aqueous phosphoric acid–MeOH (1:1) as the supercritical fluid. Real recoveries of up to 92% with relative standard deviations of less than 4.0% were achieved. These analytical techniques were also applied to assay benzophenones for ten commercial sunscreen cosmetics. We found benzophenone 3 was the most commonly used sunscreen additive in Taiwan.

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Keywords: Cosmetics; Sunscreens; Supercritical fluid extraction; Benzophenones; Tween 20

1. Introduction

Due to the use of chlorofluorocarbons (CFCs) as freezing media, the ozone layer is being destroyed gradually. Harmful UV rays shine directly to earth and cause accumulated damage to human skin, causing the skin not only to darken, but also to age more rapidly. The UV rays even act as a carcinogen [1]. Because of the damage to the ozone layer of the atmosphere, which increases harmful solar UV radiation to the earth's surface, the use of sunscreen agents has become widespread. Exposure to UV radiation produces acute or chronic effects on the skin [2]. Chemical sunscreens, which absorb damaging UV rays and so decrease the radiation, are used

widely in hair and skin cosmetic products. Benzophenone is the most commonly used sunscreen agent in cosmetics. Its recommended levels have been formulated by regulatory authorities in many countries [3–5]. Hence, the assay of sunscreen agents in commercial products is important for validation and checking their conformity to the regulation formulae.

In previous papers, ultraviolet spectrophotometry (UV) [6,7], gas chromatography (GC) [8,9], high-performance liquid chromatography (HPLC) [10–16], and even the new powerful analytical technique of capillary electrophoresis (CE) [17,18] were applied for analysis. Isolation of sunscreen agents from cosmetic matrices, prior to chromatographic analysis, requires several stages including sonication with acids, centrifugation, filtration and solvent extraction. Because these processes are laborious and time-consuming, they are not suitable for routine analyses of commercial products for which, moreover, large

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volumes of hazardous solvents must be used. Supercritical fluid extraction (SFE) is being recognized as a valuable method of extraction for its liquid-like solvating capabilities and gas-like transport properties of supercritical fluid. In addition, the dissolving power of supercritical fluid can be modified by applied pressure and/or temperature, as well as by adding a little solvent or acid. Furthermore, the most commonly used supercritical fluid, carbon dioxide, has the additional advantages of being non-flammable, non-toxic, and easily removed from the extract following decompression [19]. A previous paper by Scalia [16] described the extraction of benzophenone 3 with SFE. The reported percentage recoveries, nearly 100%, were calculated based on extraction efficiency using the solvent extraction method. Nevertheless, the recovery of spiked liquid extraction was only 75% in our investigation.

We achieved a complete study by developing an SFE method for effective pretreatment, followed by determination by HPLC and capillary zone electrophoresis (CZE) for seven benzophenones, namely: 2,4-dihydroxybenzophenone (benzophenone 1, BENZ 1), 2,2',4,4'-tetrahydroxybenzophenone (benzophenone 2, BENZ 2), 2-hydroxy-4-methoxybenzophenone (benzophenone 3, BENZ 3), 2-hydroxy-4-methoxy-5-sulfo-benzophenone (benzophenone 4,

BENZ 4), 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (benzophenone 6, BENZ 6), 2,2'-dihydroxy-4-methoxybenzophenone (benzophenone 8, BENZ 8), and 2-hydroxy-4-octyloxybenzophenone (benzophenone 12, BENZ 12) (structures as in Fig. 1), in the present study. The pK_a values of BENZ 1 and 2, with 4 and/or 4'-hydroxyl, range from 6.5 to 8.0. BENZ 4, of the sulfonyl group, is an acidic compound. The pK_a values of the other benzophenones, with 2 and/or 2'-hydroxyl, ranged from 8.5 to 10.0 [20].

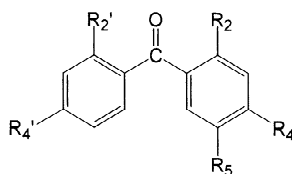
2. Experimental

2.1. Materials and reagents

BENZ 1, 3, 4, and 8 were purchased from Sigma (St. Louis, MO, USA). BENZ 2, with purity of at least 95%, was bought from the Tokyo Chemical Industry (TCI, Japan). BENZ 6 was obtained from Lancaster (UK). BENZ 12 was supplied by Aldrich (Milwaukee, IL, USA). Phosphoric acid, acetonitrile and methanol of HPLC-grade came from E. Merck (Darmstadt, Germany). Disodium tetraborate with purity of at least 99.5% was purchased from Ferak (Germany). Deionized water was purified through a purification system (Barnstead, NONA pure II) which gives a conductivity above 18 M Ω cm. Tween 20 (polyethylene sorbitan monolaurate) was obtained from Shyan-Yao (Taiwan). Commercial sunscreen products were collected from local retail stores.

2.2. HPLC

The HPLC apparatus consisted of a Perkin-Elmer 1020 LC plus integrator linked to a Model 235C diode-array detector, with 250B delivery pump with an injection valve of 100- μ l sample loop. The dual wavelengths were set at 285 and 330 nm. Separations were performed on a Nova-Pak C₁₈ column (5 μ m, 15 cm \times 4.6 mm I.D., Waters, No. 86344, USA). The optimum conditions were as follows: the equilibrium step of gradient elution was 25% acetonitrile with flow rate of 1.0 ml/min, kept for 1 min after the sample was injected, then gradient immediately to 55% for 6.7 min at a flow rate of 1.2 ml/min. Finally, the acetonitrile gradient was in-



Compounds / Abbr.	R ₂	R ₂ '	R ₄	R ₄ '	R ₅
(1) 2,4-dihydroxybenzophenone BENZ 1	OH	H	OH	H	H
(2) 2,2',4,4'-tetrahydroxybenzophenone BENZ 2	OH	OH	OH	OH	H
(3) 2-hydroxyl-4-methoxybenzophenone BENZ 3	OH	H	OMe	H	H
(4) 2-hydroxyl-4-methoxyl-5-sulfonylbenzophenone BENZ 4	OH	H	OMe	H	SO ₃ H
(5) 2,2'-hydroxyl-4,4'-dimethoxybenzophenone BENZ 6	OH	OH	OMe	OMe	H
(6) 2,2'-hydroxyl-4-methoxybenzophenone BENZ 8	OH	OH	OMe	H	H
(7) 2-hydroxyl-4-octyloxybenzophenone BENZ 12	OH	H	O-octyl	H	H

Fig. 1. Structures of the seven benzophenones studied.

creased immediately to 100% at 2.2 ml/min. The sample was filtered through a 0.45- μm syringe filter before injection into the HPLC system.

2.3. CZE

All separations were performed with a SpectraPhoresis 100 capillary electrophoresis system from Thermo Separation Products (TSP, Fremont, CA, USA) on a fused-silica capillary of 75- μm I.D. The injection mode was hydrodynamic for 0.5 s. When the capillary was first used, it was conditioned with 1.0 *M* NaOH for 50 min, then with deionized water for 25 min. The capillary was regenerated by rinsing with 0.1 *M* NaOH for 2.5 min, deionized water for 2.5 min, and a corresponding running buffer for 2.5 min. The standard solutions as well as those solutions collected from the SFE were filtered through a 0.45- μm syringe filter before they were injected into the CZE system. The optimum conditions were 0.2% Tween 20 in 20 *mM* borate buffer, 350 V/cm field strength (effective column length of 35 cm), and a monitored wavelength at 285 nm with a UV-Vis SC-100 detector. The electropherograms were recorded with an SP 400 integrator.

2.4. SFE

The cosmetic products (0.02 g) were weighed on a piece of filter paper, which was then rolled and inserted into the thimble (10 ml) and filled with sea sand (30–50 mesh, from Showa, Japan). For the extraction of fraction A, temperature and pressure were controlled at 60 °C and 3000 p.s.i., 10 min of static time and 10 ml of dynamic volume (1 p.s.i. = 6894.76 Pa). To generate supercritical fluid with 2.5% modifier (10% aqueous phosphoric acid–methanol, 1:1), a dual-pump system was used for continuous delivery for fraction B. It was controlled at 120 °C, 2500 p.s.i.. The final extract was collected with 7–8 ml acetonitrile, and then made up to 10.0 ml.

2.5. Sample treatment and preparation

For the conventional liquid extraction method, 1.00 g of cosmetic sample was weighed exactly into a centrifuge tube, 0.25 ml of 2 *M* sulfuric acid and

10 ml of methanol were added, and the tube was immersed in an ultrasonic bath thermostated at 30 °C for 30 min, then centrifuged at 5000 rpm for 20 min. The extraction procedure was repeated and the pooled supernatants were diluted to 25.0 ml with acetonitrile.

3. Results and discussion

3.1. CZE separation

Regarding the pH effect as mentioned in the Introduction, all the analytes were nearly ionized above pH 9. So, we chose a pH of 9.2 in this study. Due to the poor solubility of BENZ 12 and the inferior resolution between BENZ 2 and 4, we considered resolving these problems by adding an organic solvent and/or surfactant. An organic solvent may alter selectivity, electroosmotic mobility, and electrophoretic mobility, and increase the solubility of hydrophobic substances [21]. The addition of methanol, acetonitrile and 2-propanol from 1.0 to 10.0% were investigated. Although a certain splitting in the peak of BENZ 2 and 4 came about by adding 10% of methanol or 2-propanol, the solubility of BENZ 12 was still poor. Tween 20, a non-ionic surfactant, was found, in a previous paper [22], to enhance the solubility of a hydrophobic cationic compound. We tried adding Tween 20 between the range of 0.1% (v/v) and 0.4% at 0.1% increments and adopted 0.2% ($2.45 \cdot 10^{-7}$ *mM*) to obtain an appropriate resolution, under the CZE mode for the CMC of Tween 20 of $5.9 \cdot 10^{-5}$ *mM* [23]. The separation of analytes in a free solution was based on the different electrophoretic mobilities of the analytes, which were a function of both charge and size of the analytes. We performed the concentration of disodium tetraborate buffer from 15 to 35 *mM* at 5-*mM* increments. While the resolution was improved with the increasing buffer concentration, the operation time became longer. Since a higher electrical current would result in a higher concentration of buffer, extremely high buffer concentrations should be avoided in order to suppress Joule heat generation [23]. Consequently, 20 *mM* of borate buffer was adopted for the measurements.

Both the velocity of electrophoretic migration and

the velocity of electroosmotic flow are directly proportional to the electric field strength. Hence, higher efficiency and better resolution would be obtained by working at as high as possible field strength without producing Joule heat. In this study, we used field strengths of 320, 350, and 390 V/cm by altering the applied voltage at the fixed capillary length of 57 cm (effective length of 35 cm). Optimum separation was achieved at 350 V/cm field strength, 20 kV applied voltage. The resulting electropherogram under the optimum operation conditions is shown in Fig. 2. All the analytes were as anionic type in a buffer solution under pH 9.2, and they reversed their direction to electroosmotic flow (EOF). The migration order was in the order of charge-to-mass ratio in the CZE mode. BENZ 4, possessing the sulfonyl group, was almost ionized at pH 9.2, and it was the fastest one. Conjugation occurred in BENZ 2 and caused it to be easier to ionize than the others. On the other hand, the three compounds: BENZ 1, 6, and 8, with two hydroxyl groups, had molecular size in the order of BENZ $1 < 8 < 6$. Therefore, mobility was in the reverse order. In addition, BENZ 3 and 12, possessing one hydroxyl group only, migrated with the least mobility. The reproducibilities of migration time and peak area were determined with 10.00 $\mu\text{g}/\text{ml}$ of each standard solution, prepared with 0.2% Tween 20 in acetonitrile–water (1:1). The results are shown in Table 1. All the relative standard deviations (RSDs) of migration time and peak area were less than 2.3 and 3.5%, respectively, with five measurements. Linear ranges of the calibration curves were studied by a series of injections of standard mixture containing various concentrations of seven benzophenones. The results of linear ranges, calibration curves, and detection limits are also shown in Table 1. The determinations of detection limits were based on IUPAC method, analysis of blank solution with 20 measurements, and with a signal-to-noise ratio of 3:1.

3.2. SFE extraction

Recovery was determined by seven benzophenones spiked (0.5% of each benzophenone) in a homemade sunscreen sample in order to evaluate the proposed conditions. All the spiked recoveries

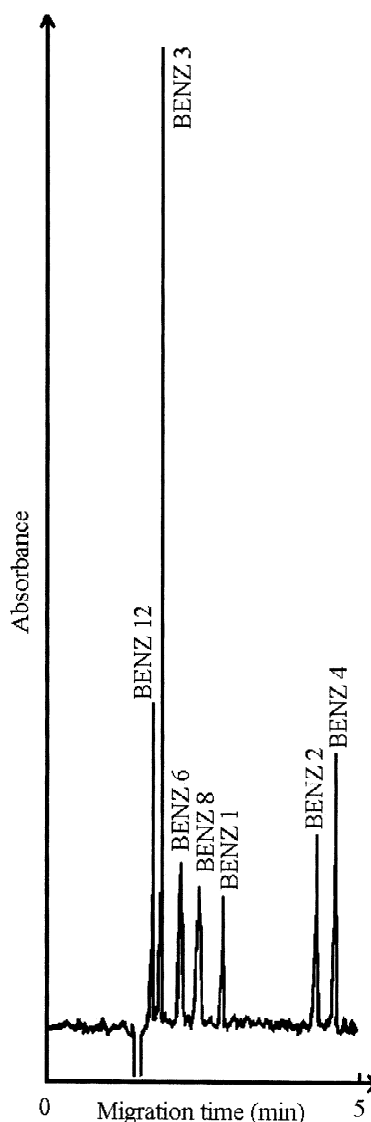


Fig. 2. Electropherogram of benzophenones and the performed conditions as described in Section 2.3.

were confirmed by HPLC–diode array detection (DAD). The solvating power of supercritical fluid increases with the temperature at a given density, and increases with the density at a fixed temperature to increase diffusion ability and to decrease viscosity [24]. Carbon dioxide, the most commonly used supercritical fluid, was also used in this study at pressures of 2000, 2500, and 3000 p.s.i., in the temperature range of 50–120 °C with 8 min of static

Table 1
Quantitative results of benzophenones by CZE^a

Item	Benzophenone						
	4	2	1	8	6	3	12
RSD of time (%)	2.04	1.69	1.92	1.97	1.77	2.26	2.21
RSD of area (%)	0.96	3.47	2.07	3.43	0.98	1.73	3.04
Linear range ($\mu\text{g/ml}$)	60.00–0.80	70.00–1.00	50.00–1.00	70.00–0.80	70.00–0.80	60.00–0.80	80.00–1.00
Slope	412.8	325.6	131.4	330.7	396.1	459.0	304.8
Intercept	133.6	64.0	274.3	195.8	106.1	69.3	129.9
Correlation coefficient	0.9995	0.9998	0.9995	0.9998	0.9993	0.9995	0.9994
Detection limit (ng/m)	614	992	845	608	709	707	843
NTP ^b ($\times 10^{-4}$)	1.97	19.44	1.01	111	2.08	3.00	3.24
Resolution	1.50				1.22		

^a The CZE conditions are described in Section 2.3 and determined with nine calibration standards and five measurements for each.

^b Number of theoretical plates.

extraction and 8.0 ml of dynamic volume (flow rate 1.0 ml/min). Nevertheless, poor recoveries of BENZ 1, 2, and 4 were obtained even though high temperature and high pressure caused their higher polarities. So, these seven benzophenones were divided into two fractions in this SFE study. Fraction A, consisting of BENZ 3, 6, 8, and 12, was performed at 3000 p.s.i. and 60 °C. We continued optimizing the static time and dynamic volume of the extraction conditions. We chose 10 min of static extraction and 8 ml of dynamic volume for 1.0 ml/min for various static times of 8–12 min and various dynamic volumes of 6–12 ml. Optimum recoveries for BENZ 8, 6, 3 and 12 of 98.6, 95.6, 96.2 and 97.5%, respectively, were obtained. We considered that the poor recoveries of fraction B, consisting of BENZ 1, 2, and 4, were due to the hydrogen-bonding interaction or van der Waals interaction between analytes of interest and matrix. We added phosphoric acid as a modifier to decrease the interaction. Satisfying recoveries, excluding BENZ 2 with four hydroxyl substituents, were obtained by adding 10% phosphoric acid–methanol (1:1) as modifier by various additions of the modifier from 0.5 to 10.0%. The hydrophobic substances of fraction A were shown to be more destitute than in the absence of a modifier. In particular, abnormal recoveries of BENZ 4 were achieved when the modifier added was over 3.0%. They were caused by impurity and were verified by over 1.2 of the purity index from data of HPLC

coupled with DAD. Nevertheless, BENZ 2 could still not be extracted even though a modifier of 10% was added. This could be considered as due to the presence of strong intermolecular hydrogen bonding. We continued to optimize the temperature, pressure, static time, and dynamic volume to enhance the recoveries of extracts. Consequently, the optimum conditions of fraction B were 120 °C and 2500 p.s.i. with 2.5% of 10% phosphoric acid–methanol (1:1), and extraction with 10 min of static time and 8 ml of dynamic volume. The high recoveries: 92.8% of BENZ 1 and 93.6% of BENZ 4, respectively, were obtained with good reproducibility of RSD, less than 4% from five extractions. In a previous paper by Scalia [16], recoveries of SFE were calculated by dividing solvent extraction efficiency, and he obtained an excellent result of complete extraction. Furthermore, from the comparison of SFE and

Table 2
The recoveries of SFE and solvent extraction

Extraction	Recovery ^a (%)						
	Benzophenone						
	4	2	1	8	6	3	12
SFE ^b	93.6	ND	92.8	98.6	95.6	96.2	97.5
Solvent ^c	24.0	91.3	119.0	81.2	81.4	74.6	51.1

^a All recoveries were the mean of five extractions.

^b The SFE conditions are described in Section 2.4.

^c The solvent extraction procedure was based on Section 2.5.

conventional solvent extraction, we were surprised by the unexpectedly poor recovery of 74.6% from the solvent extraction as shown in Table 2. This should affect the accuracy of results. However, this proposed SFE condition was able to support real content for the analysis of complex cosmetics with good accuracy.

3.3. Real sample analysis

The proposed SFE, HPLC, and CZE procedures were applied to assay benzophenones for commercial sunscreen cosmetics. Of ten real samples three showed presence of BENZ 3 only. The results are shown in Fig. 3. All of them were up to standard

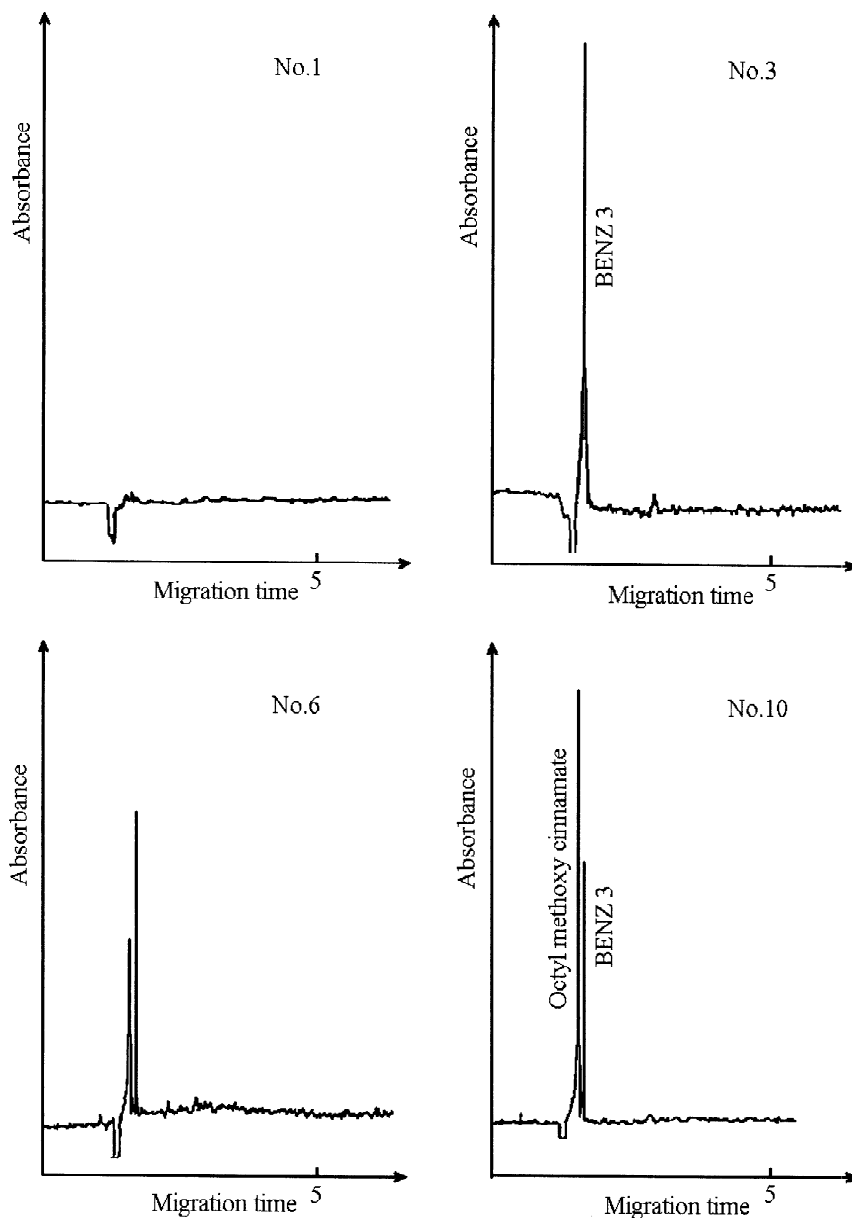


Fig. 3. Electropherograms of sunscreen cosmetics and performed conditions as described in Section 2.3.

according to the regulations for Taiwan (additions are limited to 5%). The additional peak was verified as octyl methoxycinnamate by comparison with an authentic sample. The results of HPLC and CZE showed that the SFE procedure is an effective extraction tool for complex cosmetic matrices. We confirmed the results of sample 6 by the standard addition method. It showed no significant difference between direct analysis and the standard addition at the 95% confidence level by a *t*-test, one of the statistical methods for data treatment and evaluation.

4. Conclusion

The proposed SFE, HPLC, and CZE procedures were feasible for application to an analysis of benzophenones in commercial sunscreen products. Although BENZ 2 could not be extracted by SFE, the SFE extracts of the other six benzophenones were better than by liquid extraction. Simultaneously, there was no matrix interference in the extraction of SFE. Besides, the analysis by CZE could be achieved in 5 min, and was shorter than by HPLC. Moreover, the mixture of BENZ 3 and 12 could be separated successfully by adding Tween 20 or alcohol in CZE. The mixture of BENZ 2 and 4 could be distinguished only by adding Tween 20.

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