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Original Article

Development of an LC-MS/MS Method for the Quantification of Benzophenone and its Derivatives in Settled Dust Samples

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Abstract: Benzophenone and its derivatives (BPs) are widely used in various consumer products as effective UV filters. However, they are endocrine-disrupting micropollutants with adverse effects in laboratory animals. In this work, an LC-MS/MS-based method for the determination of BP and nine of its derivatives in settled dust samples was developed. Sample preparation involved extracting with the mixture of methanol/DCM (1/1, v/v), cleaning through a SPE cartridge packed with sodium sulfate and Florisil. The target compounds were eluted with 5 mL of methanol, then concentrated under a gentle stream of nitrogen to 1 mL before transferring to a LC vial for instrumental analysis. The calibration curves of all target compounds were established in the concentration range of 0.5–500 ng/mL. The mean recoveries of target and surrogate standards ranged from 82.4 to 107%. The method quantification limit (MQL) of individual target compounds in dust samples was in the range of 0.06–0.6 ng/g.

Keywords: Benzophenone; BP-3; LC-MS/MS; Dust sample; Method development.

1. Introduction

The occurrence of these pathologies has increased due to direct exposure to sunlight. The use of sunscreens and lotions containing chemical filters (for UVA and UVB radiation) is becoming common in society today [1]. Benzophenone and its derivatives (BPs) are among the most commonly used ultraviolet filters. They work by filtering UVA and UVB radiation (290–400 nm), protecting the skin

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from sunlight by creating a thin coating on the surface. The aromatic rings used in these chemical ultraviolet filters are capable of absorbing different wavelengths from solar radiation. BPs are organic ultraviolet filters [2]. In personal care products (PCPs), BPs are often used to protect the product from ultraviolet radiation. Therefore, they can be found in other cosmetic products, such as lotions, lip balms, nail polish, shaving foam, and hairspray. During their use, BPs are released into various environments such as soil, water, air, dust, and even the food chain. Humans can be exposed to BPs through different pathways, such as inhalation, consuming food, drinking water, and dermal absorption... Recent laboratory animal studies have shown that BPs cause endocrine disruption, which is associated with hormonal changes and toxicity [3]. Therefore, BP is identified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (Group 2B), with effects on the liver and kidneys in experimental animals [4]. In PCP, the components from BP-1 to BP-12 are named according to INCI (International Nomenclature of Cosmetic Ingredients). The European Union (EU) Cosmetics Directive refers to them [5]. 2-hydroxy-4-methoxybenzophenone, also known as benzophenone-3 (BP-3), is widely used in sunscreens to block ultraviolet radiation. The concentrations currently permitted by law are insufficient to provide protection against ultraviolet radiation. They are often combined with other chemicals, such as cinnamate, camphor, and octocrylene; the concentration of each substance in the EU directly affects this combination. The EU only permits the use of BP-3 in sunscreen products. According to European Commission Regulation 1223/2009, BP-3 must be included at a level of 6% (w/w); in addition, octocrylene must be included at a level of 10% w/w, and no other BPs are permitted [6]. Octocrylene and BP-3 can decompose due to heat and ultraviolet light in the aforementioned products, leading to the formation of other BPs as a result of their high initial concentrations [7]. Endocrine effects on BP-1, -2, -3, -4, -6, and -8 have been demonstrated in several studies [2, 7, 8].

Due to using at the high levels of plastics and other materials, BP and its derivatives have been found in air, soil, water, and biological samples... However, the understanding of the distribution of BPs in living environments remains very limited. The main reason is believed to be the lack of accurate and effective methods to quantify BPs compounds in different sample matrices [3, 9]. Furthermore, regulations on permissible concentrations of BPs in environments have not been uniformly issued. Therefore, research and development of accurate analytical methods for BPs compounds in environmental samples, including dust samples, is urgent and has significant scientific implications.

There are various techniques to extract and isolate BP, referring to various pretreatment methods. The first step in the pretreatment process is to dissolve the sunscreen with a suitable solvent-often an environmentally unfriendly solvent [7]. The pre-solvent sample-dust mixture is sonically sonicated for several minutes after homogenization [5, 10, 11]. High matrix effects are a major problem with this type of sample. A hurdle for the scientific community is to minimize these effects while maintaining high extraction efficiency. The matrix often contains fats, glycerin, paraffin, and other complex components [12]. Downs et al. found BP in sunscreen without cleaning [13]. However, the pre-processing of environmental samples, including dust samples, is much more complex. This is because the concentrations of BPs in environmental samples are often low and vary greatly between sampling areas. The environmental sample matrix also contains many components that can affect the signal when measured on the device if the sample is not cleaned [9, 14-16].

Several previous studies have reported procedures for determining BP in sample matrices such as dust, water, soil, and

sediment. For water samples, recovery rates ranged from 76.6 ± 8.3% to 104 ± 6.4%, with limits of detection of 0.3–0.5 μg/L and a good linear range (1.0–24.0 μg/L) showing a very high coefficient of determination ($r^2 > 0.9969$). Excellent repeatability was also achieved, with RSD values of < 13.0% for intra-day and < 8.9% for inter-day experiments [1], Another study reported limits of quantification ranging from less than 1 to 32 ng/L, depending on the specific compound and water sample type. Recovery rates (83–105%) were unaffected by the nature of the sample matrix. [18] The total concentrations of UV filters in soil samples (500–18,380 ng/kg) and water samples (27–204 ng/L) collected in South Korea show a wide range of concentration levels [17].

This study proposes a method combining rapid and easy liquid-liquid extraction with solvent addition to the sample to extract and separate 10 BPs in settled dust samples, as well as quantification by liquid chromatography-mass spectrometry technique (LC-MS/MS). The optimized method meets the requirements for sensitivity, recovery, repeatability, and stability for accurate analysis of BP compounds in dust samples. The report also provides a preliminary assessment of the presence of several BP compounds in indoor dust samples collected from urban areas in Hanoi.

2. Experimental

2.1. Chemicals

Ten native standards including benzophenone (BP), 2,4-dihydroxybenzophenone (BP-1), 2,2',4,4'-tetrahydroxybenzophenone (BP-2), 2-hydroxy-4-methoxybenzophenone (BP-3), 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid sodium (BP-5), 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (BP-6), 2,2'-dihydroxy-4-methoxybenzophenone (BP-8), 2-hydroxy-4-(octyloxy)benzophenone (BP-12), 4-methoxybenzophenone (4-MBP), 3-(4-methylbenzylidene)camphor (4-MBC) and three surrogate standards including benzophenone-^{13}C (BP-^{13}C), 2-hydroxy-4-methoxybenzophenone-2',3',4',5',6'-*d*5 (BP-3-*d*5), (±)-3-(4-methylbenzylidene-*d*4)camphor (4-MBC-*d*4) were purchased from Laboratory of the Government Chemist LGC (ENG). HPLC grade (purities>98%) solvents, including water, acetonitrile (ACN), methanol (MeOH), and dichloromethane (DCM), were obtained from Merck KGaA (Darmstadt, Germany). The native and surrogate standards were dissolved in MeOH. Na_2SO_4 and Florisil were purchased from Merck and backed at 400 ^oC in 8 hours before use.

2.2. Method

In this report, BPs were analyzed using Shimadzu LCMS 8050 liquid chromatography-mass spectrometry (LC-MS/MS) equiped with electrospary ionization source with a Shim-pack GISS C18 100 x 2.1 mm x 1.9 μm.

Four solvent mixtures (1/1, v/v) were selected for the mobile phase to investigate their ability to separate the target substances, including (i) water (A) and acetonitrile (B), (ii) water (A) and methanol (B), (iii) acetonitrile (A) and methanol (B), and (iv) 1 mM ammonium acetate in water (A) and methanol (B).

The instrument parameters were set as follows: Flow rate: 0.3 mL/min; mobile phase solvent: (A) water, (B) MeOH; injection volume: 10 μL; column temperature: 35 °C. The mobile phase program from 0 to 2.5 min was 25% MeOH; from 2.5 to 11 min, it increased to 90% MeOH and continued to maintain until 14 min, then decreased to 25% MeOH from 14.1 to 17 min.

MS conditions were also investigated to quantify benzophenone and its derivatives (Table 3).

A blank sample was prepared by repeatedly extracting the actual sample with a 1:1 (v/v) mixture of MeOH and DCM. Recovery efficiency was determined by analyzing seven blank samples spiked with 300 ng of each analytical standard. The matrix blank was also analyzed; the results showed that the

concentrations of the substances were relatively low and negligible, having no impact on the sample analysis results.

2.3. Sample Collection

Ten indoor dust samples were collected from urban areas in Hanoi, Vietnam, during January to February 2026. Each composite sample was collected by manually sweeping floors and/or household surfaces with a non-plastic broom and a metal dustpan. The dust samples were sieved, wrapped in aluminum foil, kept in an icebox, and transported to the laboratory. Then, the samples were stored in amber glass vials at –20 ℃ until analysis.

3. Results and Discussion

3.1. The Optimal Conditions of LC-MS/MS Analysis

The results showed that the mobile phase (ii) yielded a good peak shape and peak signal. Subsequently, the gradient of the mobile phase composition was also investigated, and the optimized working conditions for separating the target compounds were selected, as shown in Table 2.

Table 2: Parameters of LC-MS/MS method for analysis of BPs

LC conditions		
Column	Shim-pack GISS C_{18} 100 mm x 2.1 mm x 1.9 µm P/N: 227-30048-02 (Shimadzu)	
Column temperature	35 ºC	
Injection volume	10 µL	
Mobile phase solvent system	(A): H_2O (B): MeOH	
Flow rate	0.3 mL/min	
Gradient of mobile phase		
Time (min)	% A: H_2O	% B: MeOH
0	75	25
2.5	75	25
11	10	90
14	10	90
14.1	75	25
17	75	25
MS/MS conditions		
Nebulizing gas flow	3.0 L/min	
Drying gas flow	10.0 L/min	
Heating gas flow	10.0 L/min	
Interface voltage	4.0 kV	
DL temperature	250 ℃	
Heat block temperature	400 ℃	
Interface temperature	300 ℃	

The fast switching polarity ionization mode method was investigated, but the results were unsatisfactory, with poor peak shape and substances that were difficult to observe.

In this study, both positive and negative ionization modes were selected for the analysis of benzophenone and its derivatives; only BP-5 was analyzed in negative ion mode, while the

remaining nine compounds were analyzed in positive ion mode.

The instrumental detection limit (IDL) is the smallest amount of compound injected into the instrument that produces a signal-to-noise (S/N) of approximately 3. In which, signal (S) was peak height at the MS/MS quantification transition, noise (N) was baseline noise in the same MS/MS transition. In this study, the instrument detection limit (IDL) was determined by injecting standard solutions at concentrations ranging from high to low until the signal-to-noise (S/N) ratio reached approximately 3; the IDL was calculated as the product of the injection volume (10 µL) and that lowest concentration. The instrument quantitation limit (IQL) was defined as three times the IDL value. The results showed that the IDL and IQL values for the target compounds ranged from 0.1–1.0 pg and 0.3–3.0 pg, respectively (Table 3). The coefficients of determination (R^2) for the calibration curves were all greater than 0.997. These results indicate that this is a promising method for the determination of benzophenone in sample matrices.

Table 3. Performance characteristics of LC-MS/MS for BP analysis

No	Name	Ion mode	Ion fragments	RT (min)	IDL (pg)	IQL (pg)	Calibration curve	R^2
1	BP	ESI+	183>105;77	11.91	0.5	1.5	y = 520790x + 3E+06	0.9974
2	BP-1	ESI+	215>137;105	11.46	0.7	2.1	y = 293537x + 541192	0.9999
3	BP-2	ESI+	247>137;109	10.08	1.0	3.0	y = 145905x + 163680	0.9998
4	BP-3	ESI+	229>151;105	12.73	0.1	0.3	y = 2E+06x + 2E+06	0.9997
5	BP-5	ESI-	307>227;211	9.11	1.0	3.0	y = 718.51x + 3349.3	0.9999
6	BP-6	ESI+	275>151;95	12.43	0.3	0.9	y = 859893x – 3E+06	0.9984
7	BP-8	ESI+	245>121;151	11.78	0.5	1.5	y = 182165x + 506258	0.9997
8	BP-12	ESI+	327>215;137	15.60	1.0	3.0	y = 282886x – 614073	0.9988
9	4-MBP	ESI+	213>105;77	12.00	0.1	0.3	y = 1E+06x + 7E+06	0.9978
10	4-MBC	ESI+	255>165;105	13.20	0.7	2.1	y = 163966x + 845544	0.9980
11	BP-^{13}C	ESI+	184>77;105	11.93	0.5	1.5	-	-
12	BP-3-d_5	ESI+	234>105;77	12.70	0.1	0.3	-	-
13	4-MBC-d_4	ESI+	259>216;108	13.70	0.7	2.1	-	-

3.2. Sample Preparation

In this report, the dust sample processing procedure has been specifically optimized as follows: Five hundred miligrams of dust samples were weighed in a 12 mL glass tube. Three hundred nanograms (300 µL of 1000 ng/mL solution) of individual surrogate standards BP-^{13}C, BP-3-*d*5, and 4-MBC-*d*4 were spiked into the sample. The target compounds were extracted by shaking with 5 mL of methanol/DCM (1/1, v/v, adjusted to pH~3 with formic acid) at 300 rpm for 20 min. Then, the sample was centrifuged at 2500 rpm for 5 min. The supernatant extract was transferred to another glass tube. The extraction was repeated two more times. The extracted solution was concentrated to ~3 mL under a gentle stream of nitrogen. Then, samples passed through a Florisil/Na_2SO_4 clean-up column containing (from bottom to top) glasswool, 0.2 g Na_2SO_4, 0.5 g Florisil, and 0.2 g Na_2SO_4, which had been conditioned with 5 mL of MeOH. The target compounds were eluted with 5 mL of methanol. The eluted solution was concentrated under a gentle

stream of nitrogen to 1 mL and transferred to a 1.5 mL amber LC-vial for analysis.

A blank procedure was also performed by adding 300 ng of each surrogate standard to a dust matrix containing no analytes to determine the recovery and trace amounts of the target substances. No target compounds were detected in the blank procedure. The matrix spiked recoveries of the target compounds were also determined by adding exactly 300 ng (300 µL of 1000 ng/mL solution) to the blank sample. The sample processing procedure was performed, and the target compounds were quantified using the LC-MS/MS method. The recoveries of the individual compound were calculated by the amount of the target compound detected in the sample according to the processing procedure, and it was added to the sample.

The method detection limit (MDL) of the target compound in the dust sample was calculated based on the instrumental detection limit (IDL), the amount of dust sample (500 mg), the volume of the concentrated sample solution (1 mL) before chromatographic analysis, and the average recovery of the target compound. The results showed that the MDL of the substances ranged from 0.02 to 0.2 ng/g. The method quantification limit (MQL) of the compounds was assigned at a value three times the MDL.

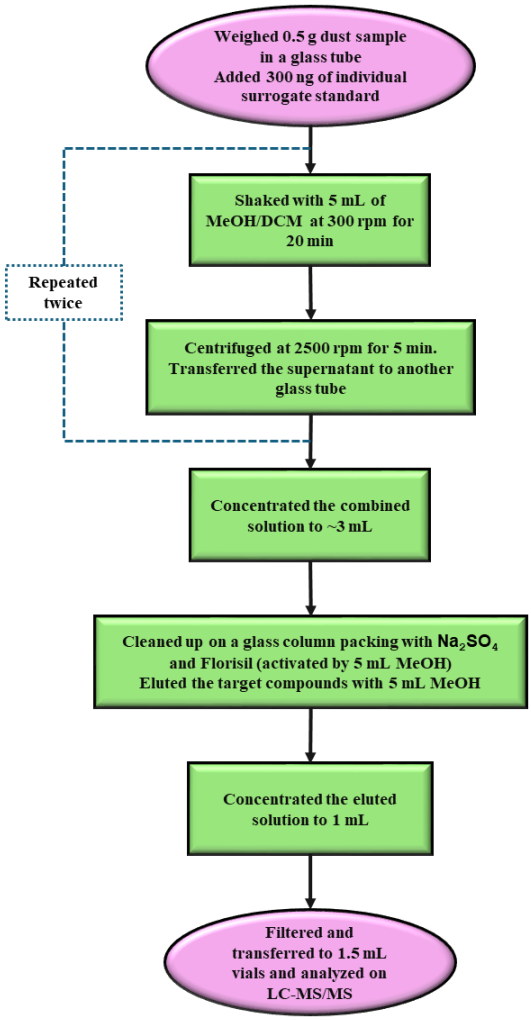

Figure 3. The treatment process of dust samples for the analysis of BPs.

Table 4. The method detection limit (MDL), quantification limit (MQL) and the recoveries of target and surrogate compounds

Compound	MDL (ng/g)	MQL (ng/g)	Recoveries (%)	
			Mean	RSD (n=7)
BP	0.1	0.3	91.1	10.1
BP-1	0.15	0.45	90.0	8.77
BP-2	0.2	0.6	82.4	10.3
BP-3	0.02	0.06	105	5.97
BP-5	0.2	0.6	89.4	5.75
BP-6	0.06	0.2	112	5.99

Compound	MDL (ng/g)	MQL (ng/g)	Recoveries (%)	
			Mean	RSD (n=7)
BP-8	0.1	0.3	88.0	7.85
BP-12	0.2	0.6	96.4	7.72
4-MBP	0.02	0.06	86.6	5.77
4-MBC	0.14	0.45	90.2	9.42
BP-^{13}C	-	-	88.3	8.83
BP3-d_5	-	-	85.8	7.07
4-MBC-d_4	-	-	87.8	7.54

The mean recovery of BP and its derivatives in dust samples with an added standard in a blank matrix ranged from 82.4 to 107%, meeting the AOAC requirement for recovery (70–120% at ppb concentration). The repeatability of the method was also quite good, assessed by RSD values ranging from 5.75 to 10.3% and meeting the AOAC requirement for repeatability with an RSD value < 15% at ppb concentration.

3.3. Concentration of BPs in Settled Dust Samples

Concentrations of benzphenone in indoor dust samples collected from Hanoi were from 5.47 to 27.9 ng/g. Among derivatives of benzophenone, BP-2, BP-5, and BP-12 were found at the ranges of <MDL–2.07, 0.09–74.5 and 3.34–16.41 ng/g, respectively. Meanwhile, other derivatives were not found in all collected samples. To our knowledge, no previous studies reported the occurrence of benzophenone and its derivatives in the environment, including dust samples, in Vietnam. The concentrations of benzophenone and its derivatives in dust samples collected in Vietnam [number of samples (*n*)=10] were lower than in other countries worldwide, such as the USA (*n*=40) (121–37400 ng/g), China (*n*=55) (9.17–3870 ng/g), Japan (*n*=22) (36.8–3360 ng/g), and South Korea (*n*=41) (31.8–8950 ng/g) [19]. Benzophenone (BP) levels in Vietnam remain low due to strict regulations under the ASEAN Cosmetic Directive, which minimize the use of endocrine-disrupting substances. The shift among Vietnamese consumers toward "clean" cosmetics and next-generation sunscreens also contributes to reducing risk, a contrast to the higher levels found in the US and South Korea, where regulatory factors and usage frequencies differ significantly from those in Vietnam.

Overall, this is the first study on developing a method for analyzing BPs in dust samples in Vietnam as well as Southeast Asian countries. However, further research with a larger sample size is needed to obtain a comprehensive assessment of BP pollution levels in the environments of Vietnam.

Table 5. Concentrations (ng/g) of BPs in dust samples

Sample	BP	BP-1	BP-2	BP-3	BP-5	BP-6	BP-8	4-MBP	4-MBC	BP-12
D.1.1	26.9	<MQL	2.07	n.d.	26.3	n.d.	<MQL	<MQL	<MQL	16.41
D.1.2	10.2	<MQL	1.69	n.d.	0.23	n.d.	<MQL	<MQL	<MQL	3.34
D.1.3	21.1	<MQL	1.05	n.d.	29.6	n.d.	<MQL	<MQL	<MQL	11.00
D.1.4	5.47	<MQL	1.18	n.d.	15.4	n.d.	<MQL	<MQL	<MQL	9.99
D.1.5	27.9	<MQL	0.19	n.d.	74.5	n.d.	<MQL	<MQL	<MQL	15.73

Sample	BP	BP-1	BP-2	BP-3	BP-5	BP-6	BP-8	4-MBP	4-MBC	BP-12
D.1.6	10.9	<MQL	0.78	n.d.	17.8	n.d.	<MQL	<MQL	<MQL	11.48
D.1.7	25.2	<MQL	0.85	n.d.	19.7	n.d.	<MQL	<MQL	<MQL	7.19
D.1.8	26.3	<MQL	0.79	n.d.	21.8	n.d.	<MQL	<MQL	<MQL	6.33
D.1.9	5.83	<MQL	<MDL	n.d.	0.09	n.d.	<MQL	<MQL	<MQL	12.28
D.1.10	24.2	<MQL	0.95	n.d.	29.5	n.d.	<MQL	<MQL	<MQL	6.88
Median	18.4	-	1.06	-	23.5	-	-	-	-	10.06
Range	5.47–27.9	-	<MDL.–2.07	-	0.09–74.5	-	-	-	-	3.34-16.41
<MQL: below the method quantification limit; n.d.: no detection										

4. Conclusions

This is the first study to report the method for the determination of BPs in dust samples using solid phase extraction combined with LC-MS/MS techniques in Vietnam. The method exhibits good recovery, suitable stability, and a low quantification limit, meeting the requirements for simultaneous analysis of BPs in dust samples. This initial study has revealed the presence of BPs in dust samples collected in Hanoi. Further research is needed to assess the distribution, exposure risks, and ecological risks from the accumulation of BPs in environmental samples in Vietnam.

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