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Development and Validation of a Rapid Method for Identification and Quantitation of Benzophenone and Related 17 Derivatives in Paper and Cardboard Packaging Materials by Gas Chromatography–Mass Spectrometry

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Benzophenone (BP) and its derivates are photoinitiators widely used in food packaging to cure inks or lacquers with ultraviolet light on cardboards and papers. Although there is no specific European legislation covering printing inks for food contact use, the European Food Safety Authority published recommendations of a limit of 0.6 mg/kg for the sum of BP and 4-methylbenzophenone. In this study, a method has been devised to test migration levels of BP and BP derivates in foodstuffs. Eleven different paper or cardboard materials were analysed for their BP and derivative contents. The qualitative and quantitative analyses of BP and other 17 derivatives for the first time were performed by gas chromatography–mass spectrometry system. The method was evaluated by validation parameters such as linearity, repeatability, accuracy and precision values. According to results, maximum values by specific migration test (at 40°C for 10 days, using TENAX) were 10.83 mg/l for BP, 0.53 mg/l for 1-hydrocyclohexylphenylketone, 0.47 mg/l for 4-methylbenzophenone, 0.22 mg/l for 2,2-dimethoxy-2-phenylacetophenone and 0.4 mg/l for methyl-2-benzoylbenzoate.

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KEY WORDS: chromatography; photoinitiator; benzophenone; paper and cardboard; migration; GC-MS

INTRODUCTION

Paper and cardboard have been used both as primary and secondary packaging for a wide range of foods for many years and represent a large and constantly growing part of the food packaging industry because of their advantages such as easy treatment and favourable cargo advantages compared with other traditional packaging materials.[1] In the area of food packaging, benzophenone (BP) is widely used as photoinitiator (PI) for inks and lacquers that are cured with ultraviolet (UV) light.[2] The role of PI is to start the polymerization processing in order to harden the ink film to the substrate.[3,4] Traditionally, inks were cured thermally, and their formulations included organic solvents, which then had to be eliminated with a drying process step.[3] PIs such as BP are not completely used up or removed during or after the printing process. Even though the inks and lacquers are applied to the outside of the packaging material, low molecular weight substances like BP can permeate through the rather open structure of cardboard and subsequently can migrate foodstuffs by mass transference packaged in these materials.[5,6] So, migration of PIs from the packaging to the food through the vapour phase is possible even from the secondary packaging, i.e. when the ink is not in direct contact with the food.[7–9] The rate of the migration of PIs through the vapour phase is highly influenced by their boiling point and the vapour pressure as well as by the type of the food package.

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Benzophenone may also be present in recycled paper or cardboard if the recycling process has not fully stripped the substances above from the recovered paper.[2] Then BP may persist in any packaging made from these recycled materials even if that packaging itself has not been printed with UV-cured inks containing BP or any derivatives.[2,3] As the paperboard of secondary packaging is widely printed with the UV inks, it is important to consider that the most commonly used raw material for paperboard is recycled, and the levels of PIs, such as BP, might persist or even accumulate in recycled board.[10] Recycled board is commonly used in direct contact not only with dry foodstuffs, such as flour and pasta, but also with fast-food items, i.e. short duration contact food types, such as pizzas.[10]

Although there is no specific European Union control for migration from inks to foodstuffs, there is a Group Tolerable Daily Intake for BP of 0.01 mg/kg of body weight. Moreover, for the use of BP as an additive in plastic, there is a specific migration limit (SML) of 0.6 mg/kg according to the European Commission Directive 2002/72/EC and Commission Regulation 10/2011.[3]

Specific migration studies have shown that BP can migrate from paper and board to dry foods[1,8,9] or powders simulating dry foods.[11] Because of its volatility, BP may migrate through paperboard to the food if no effective barrier is present.[7,12,13,8]

Previous laboratory studies of Johns *et al.*[14] showed that BP could be present in cardboard at levels up to 0.7 mg/dm^2.[7] The studies also indicated that BP migrates readily to foods with levels in foods stored in cardboard at −20°C ranging up to 0.4 mg/kg. The extent of migration into foods stored under these conditions and subsequently heated in the packaging was shown to increase, with levels after heating ranging up to 1.0 mg/kg.[15] This indicated that BP can migrate to foods, even during frozen storage.[7]

Some studies have focused on method development to determine the different kinds of PIs.[4,7–9,16,17,10,18] Different gas chromatography–mass spectrometry (GC-MS) methods were developed or devised and widely used to analyse the BP extract; otherwise, some high-performance liquid chromatography (HPLC) methods were used for the determination of BP and related derivatives.[3,7,10] The optimization and validation of methods to determine potential low-weight substances used in packaging formulations for food are essential to ensure the safety of packaged foods. Nevertheless, this is not easy to achieve because food samples are complex matrices. There is little information available regarding the determination of PIs, especially in food items. Most of the PI determination analysis is achieved by using packaging material[5] or using a food simulant.[3,6]

The aim of the present paper is to develop a rapid method for the qualitative and quantitative determination of migration of BP and 17 derivatives from paper and cardboard packaging materials to food simulants by using GC-MS and to determine migration level of PIs in foods from selected packaging materials. In literature, there are some studies that investigate some BP derivatives; however, in this study, almost all the BP derivatives were investigated all together for the first time. For this purpose, 11 samples of paper and cardboard (seven recycled cardboards and four virgin papers) were purchased from local paperboard or cardboard suppliers and retail markets in Turkey.

MATERIAL AND METHOD

Chemical and standards

All chemicals (solvents and organic pollutants) were of analytical grade and purchased from Sigma (Taufkirchen, Germany) and Fluka (Buchs, Switzerland). Standard benzophenone, 2-methylbenzophenone, 1-hydrocyclohexyl phenyl ketone, ethyl-4-dimethylaminobenzoate, *N*-ethyl-p-toluene sulfonamide, 2-hydroxybenzophenone, 3-methylbenzophenone, 4-methylbenzophenone, 2,2-dimethoxy-2-phenylacetophenone, methyl-2-benzoylbenzoate, 4-fluoro-4-hydroxybenzophenone (internal standard), 4-hydroxybenzophone, flavone (internal standard), 2-ethylhexyl-4-benzophenone, 2-methyl-4-(methylthio)-2-morpholinopropiophenone, 4-ısopropylthioxanthone, 2-isopropylthioxanthone, 4-benzoylbiphenyl, 2,2-diethyl-9H-thioxanthen-9-one and 4,4-bis(diethylamino)benzophenone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol and diethyl ether were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the standards used had purity levels above 98%. Physicochemical information about these standard PIs is included in Table 1. All solvents were

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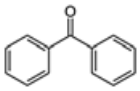
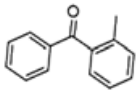
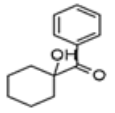
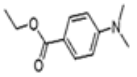
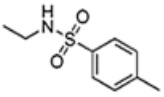
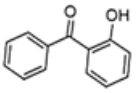
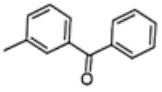
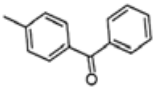
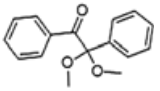
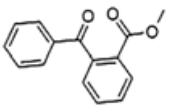
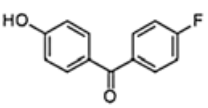
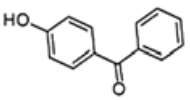
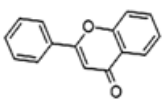
Table 1. Physicochemical information about the selected standard benzophenone and other 17 derivatives and two internal standards.[15,19]

Standard name	Cas no	Molecular Weight (M.W)	b.p./m.p.[1] (°C)	Molecular formula	Structure
Benzophenone (diphenylmethanone)	119-61-9	182.2	305/49	$C_{13}H_{10}O$	
2-Methylbenzophenone	131-58-8	196.2	126/−18	$C_{14}H_{12}O$	
1-Hydrocyclohexyl phenyl ketone	947-19-9	204.6	175/47 − 50	$C_{13}H_{16}O_2$	
Ethyl-4-dimethylaminobenzoate	10287-53-3	193.2	190/65	$C_{11}H_{15}NO_2$	
N-ethyl-p-toluene sulfonamide	80-39-7	199.2	208/64	$C_9H_{13}NO_2S$	
2-Hydroxybenzophenone	117-99-7	198.2	172/38	$C_{13}H_{10}O_2$	
3-Methylbenzophenone	643-65-2	196.2	184	$C_{14}H_{12}O$	
4-Methylbenzophenone	134-84-9	196.2	326/57	$C_{14}H_{12}O$	
2,2-Dimethoxy-2-phenylacetophenone	24650-42-8	256.3	169/68	$C_{16}H_{16}O_3$	
Methyl-2-benzoylbenzoate	606-28-0	240.2	352/51	$C_{15}H_{12}O_3$	
4-Fluoro-4-hydroxybenzophenone (IS)	25913-05-7	216.2	–	$C_{13}H_9FO_2$	
4-Hydroxybenzophone	1137-42-4	198.2	150160/132135	$C_{13}H_{10}O_2$	
Flavone (IS)	525-82-6	222.2	–	$C_{15}H_{10}O_2$	

(Continues)

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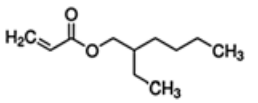
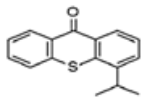
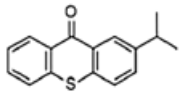
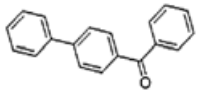
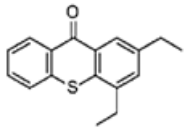
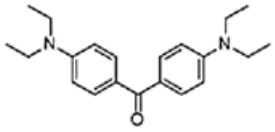
Table 1. (Continued)

Standard name	Cas no	Molecular Weight (M.W)	b.p./m.p.[1] (°C)	Molecular formula	Structure
2-Ethylhexyl-4-(dimethylamino) benzophenone	21245-02-3	276.4	–	$C_{17}H_{26}NO_2$	
2-Methyl-4-(methylthio)-2-morpholinoprophenone	71868-10-5	279.4	–/75	$C_{15}H_{21}NO_2S$	
4-Isopropylthioxanthone	83846-86-0	254.3	–	$C_{16}H_{14}OS$	
2-Isopropylthioxanthone	5495-84-1	254.3	210/76	$C_{16}H_{14}OS$	
4-Benzoylbiphenyl	2128-93-0	258.3	419/100	$C_{19}H_{14}O$	
2,2-Diethyl-9H-thioxanthen-9-one	82799-44-8	268.3	68	$C_{17}H_{16}OS$	
4,4-Bis(diethylamino)benzophenone	90-93-7	324.4	151/90	$C_{21}H_{28}N_2O$	

[1]Average value.

of HPLC grade. Modified polyphenylene oxide (MPPO), 60 to 80 mesh is also known as TENAX used as a food simulant.

Samples

Eleven different paper and cardboard packaging samples (seven recycled and four virgin packaging) used in this study were supplied by cardboard or paper suppliers in Turkey. All the packaging samples had different properties such as type, grammage and thickness. Some physical properties of the packaging samples were given in Table 2.

Methods

Sample preparation and analysis of packaging materials.

Preparation of standard solutions and internal standard. For each BP and the other 17 derivatives, a stock solution was prepared by dissolving 100 mg of standards in 25 ml of pure methanol for 4000 mg/l. Standard solutions used for calibration curve were prepared by diluting stock solutions with pure methanol at different concentrations in the range of 0.1–2.0 mg/l in triplicate ($n = 3$). Then the solutions were given to the GC-MS system as shown in Figure 1. Solutions were stored in a refrigerator in the dark before the analysis. The solution of the internal standard flavone and 4-fluoro-4-hydroxybenzophenone used to spike the samples was prepared in methanol at a concentration of 0.6 mg/l, which is the preferred concentration of internal standards in the extracts to be determined by GC-MS.

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Table 2. Some physical properties of paper and cardboard samples.

Code	Type	Recycled (%)	Grammage (g/m^2)	cm^3/g	Thickness (μm)
RP 1		87–93	225	1.11	250
RP 2		100	225	1.22	275
RP 3	Multicolor special	100	230	1.22	280
RP 4		95–97	225	1.20	270
RP 5		100	250	1.24	310
RP 6	Metallized paper	100	357	1.26	450
RP 7	Fully coated white lined chipboard	100	280	1.37	380
VP 1	Fully coated boxing board	0	500	1.88	940
VP 2		0	235	1.57	370
VP 3	Hi-bulk structure	0	235	1.51	355
VP 4	Fully coated bleached paperboard with white back	0	285	1.70	485

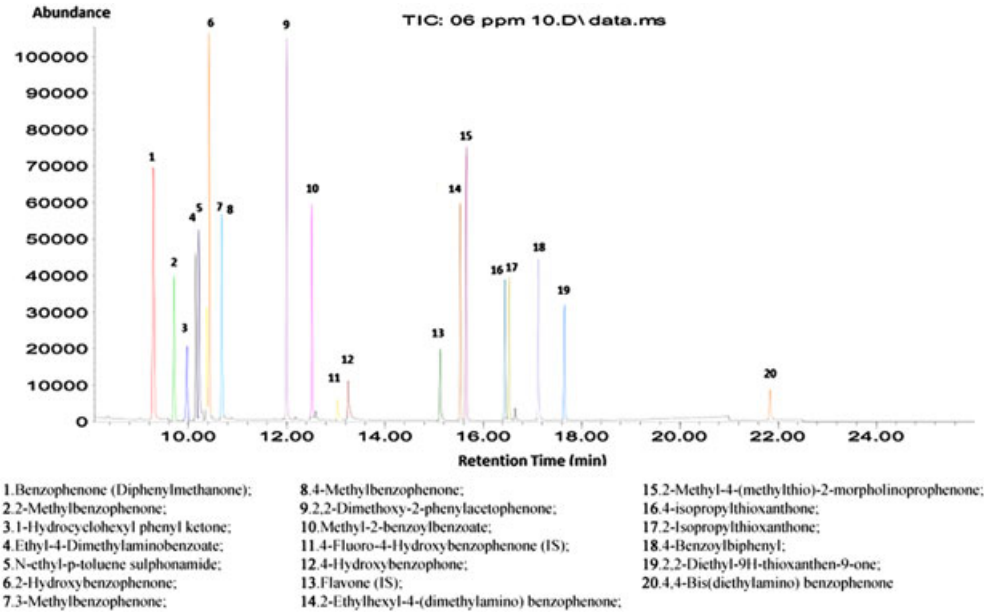

Figure 1. Gas chromatography–mass spectrometry chromatogram of standard benzophenone and other 17 derivatives.

Preparation of TENAX

For extraction of TENAX before usage, using acetone is obligatory for complete purification of the MPPO prior to the first use in this test procedure. MPPO was placed in a Soxhlet cartridge and extracted for 6 h with diethyl ether. MPPO was spread in a Petri dish of suitable diameter, and the Petri dish was placed in a fume hood. The solvent was evaporated while frequently mixing with a glass rod. The Petri dish was placed in an oven at 160°C for 6 h. After heating, MPPO was ready to use and stored.

Sample preparation

The test specimen was prepared by cutting 1 dm^2 area with the cutting implement. After that, the test specimen was placed in a Petri dish, and 4 g MPPO was evenly placed in the Petri dish. The Petri dish was put into an oven, which is preheated to the test temperature at 40°C. After 10 days, MPPO was transferred to a 100 ml Erlenmeyer flask; 30 ml diethyl ether solution was added and then shook manually for 1 min. The Erlenmeyer flask was allowed to stand for 5 min without shaking. The solvent

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was decanted from the MPPO extraction through the filter into the flask. This extraction procedure was repeated twice using 20 ml solvent. All extraction solvent was evaporated to dryness on a steam bath. The Erlenmeyer flask was hold at room temperature for cooling down. Five millilitres acetonitrile was poured into the Erlenmeyer flask and vortexed for 1 min. After that, the extraction solvent was filtered through a 0.45 μm polytetrafluorethylene filter and transferred into a 2 ml vial to be injected for GC-MS analysis.

Chromatographic conditions

Gas chromatography–mass spectrometry analysis. The qualitative and quantitative analyses of benzophenone and other 17 derivatives were performed by GC-MS system, which is equipped with a gas chromatography (Agilent Technologies 7890A), mass spectroscopy (Agilent Technologies 5975C) and an autosampler (Agilent Technologies 7693A). A published method of Lloyd[20] with minor modifications was used to analyse the cardboard and paper samples. The injector port was EPC split/splitless (Agilent Technologies 7890A), and injections were carried out in a splitless injector at 280°C. An injection volume of 1 μl in hot splitless mode was used. Purge flow to split vent was 50 ml/min at 0.75 min. The carrier gas was helium with a flow of 0.3 ml/min. Inlet pressure was 17.0 psi. Oven program was at 100°C for 1 min, and then the oven was programmed to rise at 10° C/min to 300°C, where it was held for 5 min. The analysis was carried out using an HP5MS column (30 m × 250 μm × 0.25 μm). Detection was carried out in selected ion monitoring mode. Ion source temperatures were set at 250°C, and a solvent delay of 8 min was applied.

RESULTS AND DISCUSSION

Rapid method validation

The validation performance characteristics were chosen for the assay method type. Validation of the GC-MS method for analysis of BP and selected other 17 derivatives was achieved by investigating the chosen parameters: linearity, limit of detection, limit of quantification, repeatability (intra-day and inter-day precision), accuracy and recovery.[21–24] To assert that the GC-MS method had performances compatible with those required for routine analysis of BP compounds from paper and cardboard samples, a rapid validation of this method (without ruggedness study) was carried out as shown in Table 3.

Linearity and working range

Linearity of the method was evaluated by injecting five different concentrations of standard mixture prepared in methanol in the range of 0.1–2.0 mg/l in triplicate ($n = 3$) for 3 days, keeping the injection volume constant. A calibration curve was prepared from the standard solutions to confirm a linear relationship between the peak area ratio and the concentration BP and other 17 derivatives. If the correlation coefficient is $R^2 \geq 0.995$, the method is accepted as linear. All compounds showed good linearity (>0.995), and the relative standard deviations (RSDs) ranged from 0.69% to 4.57% for day-to-day precision. The linearity ranges and linear regression equations of each standard compound were given in Table 4.

As seen in Table 4, all of the standard PIs exhibited good linearity, and the concentration-peak area correlation coefficients (R^2) were greater than 0.995. The expected good results were obtained from this validation parameter of our method. The lowest RSD value was found for 4-benzoylbiphenyl at a level of 1.18%, whereas the highest RSD value was found for 3-methylbenzophenone at a level of 3.63%.

Accuracy and precision

Accuracy was reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and accepted true value together with concentration of measurement made. The standard deviation (SD) and RSD shall be reported for each type of precision

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Table 3. Retention time and characteristic ions used for the GC-MS detection of the BP and other 17 derivatives.

No	Standards/photoinitiators	Retention time[1] (min)	Target ion and qualifier ions (m/z)
1	Benzophenone (diphenylmethanone)	9.285	105-77-182
2	2-Methylbenzophenone	9.7063	195-196-347
3	1-Hydrocyclohexyl phenyl ketone	9.969	99-81-77
4	Ethyl-4-dimethylaminobenzoate	10.152	148-193-164
5	*N*-ethyl-p-toluene sulfonamide	10.211	91-155-184
6	2-Hydroxybenzophenone	10.372	197-198-121
7	3-Methylbenzophenone	10.42	119-196-105
8	4-Methylbenzophenone	10.6783	119-196-91
9	2,2-Dimethoxy-2-phenylacetophenone	12.001	151-105-77
10	Methyl-2-benzoylbenzoate	12.512	163-105-77
11	4-Fluoro-4-hydroxybenzophenone (internal standard)	13.03	121-216-123
12	4-Hydroxybenzophone	13.252	121-198-77
13	Flavone (internal standard)	15.12	222-120-194
14	2-Ethylhexyl-4-(dimethylamino)benzophenone	15.534	165-277-148
15	2-Methyl-4-(methylthio)-2-morpholinoprophenone	15.654	128-42
16	4-isopropylthioxanthone	16.4409	239-254-240
17	2-Isopropylthioxanthone	16.524	105-77-182
18	4-Benzoylbiphenyl	17.1227	195-196-347
19	2,2-Diethyl-9H-thioxanthen-9-one	17.6534	99-81-77
20	4,4-Bis(diethylamino)benzophenone	21.8375	148-193-164

[1]Data are mean values from triplicates.

Table 4. The linearity ranges and linear regression equations of each standard BP and related derivatives.

Standards/photoinitiators	Slope *(m)	y-intercept *(*n*)	Coefficient of regression*
Benzophenone (diphenylmethanone)	121 142.30	4130.46	0.9995
2-Methylbenzophenone	89 062.01	−2744.61	0.9997
1-Hydrocyclohexyl phenyl ketone	76 118.09	−6332.34	0.9989
Ethyl-4-dimethylaminobenzoate	72 908.05	−2273.26	0.9994
N-ethyl-p-toluene sulfonamide	61 519.76	1366.20	0.9996
2-Hydroxybenzophenone	70 019.24	−5449.03	0.9986
3-Methylbenzophenone	105 767.49	−518.97	0.9991
4-Methylbenzophenone	97 970.72	−4260.33	0.9996
2,2-Dimethoxy-2-phenylacetophenone	172 743.97	−7032.60	0.9997
Methyl-2-benzoylbenzoate	91 500.06	−4519.27	0.9991
4-Hydroxybenzophone	1.61	0.08	0.9990
2-Ethylhexyl-4-(dimethylamino)benzophenone	3.85	−0.62	0.9983
2-Methyl-4-(methylthio)-2-morpholinoprophenone	7.20	−1.00	0.9990
4-Isopropylthioxanthone	2.31	−0.42	0.9984
2-Isopropylthioxanthone	2.63	−0.51	0.9986
4-Benzoylbiphenyl	2.59	−0.52	0.9990
2,2-Diethyl-9H-thioxanthen-9-one	1.86	−0.24	0.9989
4,4-Bis(diethylamino)benzophenone	2.23	−0.50	0.9987

*Data are mean values from triplicates.

investigation. Each 0.6 mg/L sample spike of BP and related derivatives, which means 0.6 mg/l concentration of these compounds was put in sample for three different days, was carried out on 10 replicates with GC-MS. The obtained values were 71.28% for BP and ranged between 70.33% and 106.29% for the other 17 derivatives. If the %average RSD $\leq$ 5%, the method has acceptable precision, and if the area count of all the replicates was close to each other (RSD $<$ 5%), the method has acceptable accuracy. The recovery was generally close to 100% and higher than $>$70%, except for 4-benzoylbiphenyl that shows an average recovery rate of 66.27%, 3-methylbenzophenone with 60.77% and 4-hydroxybenzophenone with 50.51%, in all the cases. However, it can be concluded that all the compounds showed good accuracy and precision as seen in Table 5.

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Table 5. Recovery of the standard BP and related derivatives by GC-MS method.

Compound	Analysis	Average concentration[1]	SD	Average SD (%)	% Recovery	Average recovery rate (%)	% RSD	Average RSD (%)
Benzophenone	Trial 1	0.4270	1.8151	1.1111	71.16	71.28	2.55	1.56
	Trial 2	0.4245	0.5418		70.75		0.77	
	Trial 3	0.4316	0.9764		71.93		1.36	
2-Methylbenzophenone	Trial 1	0.4216	1.6844	1.0338	70.26	70,33	2.40	1.47
	Trail 2	0.4195	0.4977		69.92		0.71	
	Trail 3	0.4249	0.9193		70.81		1.30	
1-Hydrocyclohexyl phenyl ketone	Trial 1	0.5719	2.76	1.6162	95.31	95.68	2.90	1.69
	Trail 2	0.5682	0.79		94.70		0.83	
	Trail 3	0.5822	1.30		97.03		1.34	
Ethyl-4-dimethylaminobenzoate	Trial 1	0.517	1.970276	1.2991	86.13	87.24	2.29	1.49
	Trail 2	0.521	0.879649		86.85		1.01	
	Trail 3	0.532	1.047448		88.74		1.18	
N-ethyl-p-toluene sulfonamide	Trial 1	0.581	4.24	3.4328	96.81	93.58	4.38	3.66
	Trail 2	0.551	2.89		91.79		3.15	
	Trail 3	0.553	3.16		92,13		3.43	
2-Hydroxybenzophenone	Trial 1	0.537	0.829621	1.2354	89.55	87.98	0.93	1.41
	Trail 2	0.518	1.451265		86.35		1.68	
	Trail 3	0.528	1.425165		88.05		1.62	
3-Methylbenzophenone	Trial 1	0.414	3.15	2.2464	68.94	60.77	4.57	3.63
	Trail 2	0.367	1.97		61.17		3.22	
	Trail 3	0.313	1.62		52.20		3.10	
4-Methylbenzophenone	Trial 1	0.476	1.246084	0.9917	79.41	80.05	1.57	1.24
	Trail 2	0.479	0.722022		79.85		0.90	
	Trail 3	0.485	1.007133		80.87		1.25	
2,2-Dimethoxy-2-phenylacetophenone	Trial 1	0.547	1.64	1.0911	91.08	91.51	1.80	1.19
	Trail 2	0.545	0.63		90.85		0.69	
	Trail 3	0.556	1.01		92.60		1.09	
Methyl-2-benzoylbenzoate	Trial 1	0.636	2.31653	1.6351	106.06	106.29	2.18	1.54
	Trail 2	0.636	1.159555		106.07		1.09	
	Trail 3	0.640	1.429159		106.73		1.34	
4-Hydroxybenzophone	Trial 1	0.310	1.40	0.8901	51.71	50.51	2.70	1.75
	Trail 2	0.308	0.60		51.27		1.17	
	Trail 3	0.291	0.67		48.56		1.38	
2-Ethylhexyl-4-(dimethylamino) benzophenone	Trial 1	0.517	3.35	2.0629	86.16	81.22	3.88	2.50
	Trail 2	0.483	1.59		80.58		1.97	
	Trail 3	0.461	1.25		76.91		1.63	
2-Methyl-4-(methylthio)-2-morpholinoprophenone	Trial 1	0.474	2.91	1.7223	79.08	74.70	3.67	2.26
	Trail 2	0.446	1.36		74.40		1.83	
	Trail 3	0.424	0.90		70.63		1.27	
4-Isopropylthioxanthone	Trial 1	0.449	2.75	1.3198	74.83	72.07	3.68	1.80
	Trail 2	0.431	0.60		71.83		0.84	
	Trail 3	0.417	0.61		69.57		0.87	
2-Isopropylthioxanthone	Trial 1	0.448	2.56	1.2844	74.68	71.96	3.43	1.75
	Trail 2	0.427	0.68		71.22		0.95	
	Trail 3	0.420	0.62		69.98		0.88	
4-Benzoylbiphenyl	Trial 1	0.403	1.56	0.7854	67.09	66.27	2.32	1.18
	Trail 2	0.394	0.24		65.74		0.36	
	Trail 3	0.396	0.56		65.97		0.85	
2,2-Diethyl-9H-thioxanthen-9-one	Trial 1	0.452	2.82	1.2851	75.41	72.84	3.73	1.73
	Trail 2	0.432	0.59		72.07		0.82	
	Trail 3	0.426	0.45		71.04		0.63	
4,4-Bis(diethylamino)benzophenone	Trial 1	0.597	3.78	2.7927	99.49	101.59	3.80	2.76
	Trail 2	0.621	1.65		103.48		1.60	
	Trail 3	0.611	2.94		101.81		2.89	

[1]Data are mean values from ten replicates.

Limit of detection and limit of quantification

Limit of detection (LOD) is defined as the lowest amount of analyte that can be detected above baseline noise. It is calculated from a formula constructed as $3 \times S/N$ (three times of signal-to-noise ratio) or detection limit shall be assessed by analysing 10 independent sample blanks fortified at the lowest acceptable concentration and calculated by a formula constructed as $3 \times SD$. Limit of quantification (LOQ) is also defined as the lowest amount of analyte that can be quantified reproducibly above the baseline noise. It is calculated from a formula constructed as $10 \times S/N$ (10 times of signal-to-noise ratio) or a formula constructed as $10 \times SD$.[25] Below the limit of quantification value,

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a method can only produce semi-quantitative or qualitative data, but quantitation of the analyte cannot be achieved.[26,27] Ten replicate determinations of 0.1 mg/l standard BP and each BP derivative were performed for LOD and LOQ by using GC-MS. LOD and LOQ values of studied BP and related derivatives were given in Table 6.

Repeatability

Repeatability is defined as the precision under the same operating conditions over a short interval of time. A single standard solution at 0.6 mg/l concentration was prepared, 10 subsequent injections were performed and deviation was calculated. Each sample was prepared separately. The repeatability (intra-day and inter-day precision or within run and between run) of the GC-MS method was studied by determining the variation in retention time and area of the peak of the ion and their RSD% and results were given in Table 7. When RSD% $< 5\%$, the result is accepted as appropriate. RSD values of the method were between 1.18% and 3.88% as shown in Table 7.

Evaluation of migration levels

Eleven samples were selected at random from the cardboard suppliers; seven of them were recycled, and the four virgin packages were analysed for BP and other 17 derivatives content. Table 8 gives the mean migration level results of BP and other derivatives obtained with single-side contact of uncontaminated paperboard samples with TENAX using the test conditions of 10 days at 40°C. BP was detected in seven of the 11 packaging samples analysed as present in Table 8. All the recycled cardboard packaging samples found migrated, exceeding the SML 0.6 mg/kg. Migration level of BP in these samples ranged from 1.41 to 10.83 mg/kg. The highest migration level of BP was determined in the sample RP2. BP was the most abundant UV initiator found in this survey and was determined in quantitative levels in seven samples, whereas 1-hydrocyclohexyl phenyl ketone was found in only one sample as seen in Table 8. When the results of our study were compared with a comprehensive laboratory study of Anderson and Castle[7] in their study, the food contained in 71 packages was analysed for BP.[7] BP was detected in 51 of the 71 food samples (72%) analysed. Twenty samples

Table 6. LOD and LOQ values of the GC-MS method for analysis of standard BP and other 17 derivatives (assayed concentration 0.1 mg/l).

Standards/photoinitiators	Average area	LOD (3 × SD)[1] (area)	LOQ (10 × SD) [1] (area)	LOD	LOQ
Benzophenone (diphenylmethanone)	14 056.4	1507.56	5025.21	0.011	0.036
2-Methylbenzophenone	7973.7	585.95	1953.16	0.007	0.024
1-Hydrocyclohexyl phenyl ketone	4261.7	1735.1	5783.68	0.041	0.136
Ethyl-4-dimethylaminobenzoate	5639.2	568.33	1894.44	0.010	0.034
N-ethyl-p-toluene sulfonamide	8244.3	865.45	2884.85	0.010	0.035
2-Hydroxybenzophenone	4081.9	907.14	3023.81	0.022	0.074
3-Methylbenzophenone	63 196.8	8134.84	27 116.13	0.013	0.043
4-Methylbenzophenone	7730.6	1501.07	5003.56	0.019	0.065
2,2-Dimethoxy-2-phenylacetophenone	13 375.8	1024.19	3413.97	0.008	0.026
Methyl-2-benzoylbenzoate	6735.4	964.8	3215.99	0.014	0.048
4-Hydroxybenzophone	2769.4	4887.48	16 291.6	0.176	0.085
2-Ethylhexyl-4-(dimethylamino) benzophenone	7958.6	708.34	2361.14	0.009	0.043
2-Methyl-4-(methylthio)-2-morpholinoprophenone	14 515.8	3306.92	11 023.08	0.023	0.051
4-Isopropylthioxanthone	4322.1	1029.64	3432.13	0.024	0.059
2-Isopropylthioxanthone	4781.8	2242.6	7475.33	0.047	0.036
4-Benzoylbiphenyl	4332.9	769.96	2566.53	0.018	0.039
2,2-Diethyl-9H-thioxanthen-9-one	4621.6	518.75	1729.15	0.011	0.179
4,4-Bis(diethylamino)benzophenone	1660.1	510.11	1700.38	0.031	0.080

LOD, limit of detection; LOQ, limit of quantification; GC-MS, gas chromatography-mass spectrometry.
[1]Data are mean values from ten replicates.

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Table 7. Mean values of average area amount and retention time of BP and related derivatives and their % values for repeatability of the method.

Standards/photoinitiators	Average area[1]	SD	%RSD	Average RT[1]	SD	%RSD
Benzophenone	74 613.0	1912.06	2.56	9.28	0.002	0.023
2-Methylbenzophenone	49 627.7	555.00	1.11	9.70	0.002	0.018
1-Hydrocyclohexyl phenyl ketone	21 287.5	670.28	3.14	9.97	0	0
Ethyl-4-dimethylaminobenzoate	36 011.2	784.90	2.18	10.15	0	0
N-ethyl-p-toluene sulfonamide	36 861.1	574.71	1.56	10.21	0	0
2-Hydroxybenzophenone	25 616.6	996.03	3.88	10.37	0	0
3-Methylbenzophenone	50 688.7	938.63	1.85	10.42	0	0
4-Methylbenzophenone	52 934.2	728.72	1.37	10.67	0	0.005
2,2-Dimethoxy-2-phenylacetophenone	91 752.0	1206.75	1.31	12.00	0	0
Methyl-2-benzoylbenzoate	44 615.2	1508.45	3.38	12.51	0	0
4-Hydroxybenzophone	18 193.8	641.74	3.52	13.25	0.003	0.021
2-Ethylhexyl-4-(dimethylamino) benzophenone	56 835.7	1172.25	2.06	15.53	0	0
2-Methyl-4-(methylthio)-2-morpholinoprophenone	108 197.9	1362.52	1.26	15.654	0.002	0.013
4-Isopropylthioxanthone	33 383.3	471.67	1.41	16.44	0.003	0.019
2-Isopropylthioxanthone	37 986.1	489.99	1.29	16.52	0	0
4-Benzoylbiphenyl	37 367.9	465.49	1.24	17.12	0	0.003
2,2-Diethyl-9H-thioxanthen-9-one	28 478.8	380.89	1.33	17.65	0.002	0.01
4,4-Bis(diethylamino)benzophenone	16 234.5	546.17	3.36	21.83	0.003	0.014

RT, retention time; SD, standard deviation; RSD, relative standard deviation.
[1]Data are mean values from triplicates.

(28%) were higher than 0.5 mg/kg, and the highest level of BP in food was 7.3 mg/kg. Koivikko *et al.*[15] found BP and 4-MBP in quantitative levels in 27 samples (59%) of the total 46 samples, and the level of BP was between 0.02 and 3.99 mg/kg, whereas 4-MBP was found in 14 samples (30%), while the highest level of it was 4.41 mg/kg.[15] In our study, we obtained higher level of BP migration (10.83 mg/kg) in the samples collected from Turkish paper and cardboard suppliers and markets. Moreover, measurable levels of 4-methylbenzophenone were found in four samples RP3, RP5, RP6 and RP7, where the levels of the compound were 0.47, 0.34, 0.24 and 0.47 mg/kg, respectively. However, migration levels of 4-methylbenzophenone were lower than the results of Koivikko *et al.*[15] and were under the recommendation limit of EFSA, which is an SML of 0.6 mg/kg.

Methyl-2-benzoylbenzoate was determined in two samples, one virgin and one recycled sample at the levels of 0.40 and 1.41 mg/kg, respectively. This chemical was the only BP derivative found in the virgin paper sample. BP and other derivatives were not present in the same sample. 2,2-Dimethoxy-2-phenylacetophenone was also found in two samples at the levels of 3.58 and 0,22 mg/kg for the RP1 and RP7 samples, respectively.

Table 8. Specific migration levels (mg/kg) of test samples at 40°C for 10 days by using TENAX simulant.

Packaging material	Benzophenone	1-Hydrocyclohexyl phenyl ketone	4-Methylbenzophenone	2,2-Dimethoxy-2-phenylacetophenone	Methyl-2-benzoylbenzoate
RP 1	5.31	N.D.	N.D.	3.58	1.41
RP 2	10.83	N.D.	N.D.	N.D.	N.D.
RP 3	1.94	N.D.	0.47	N.D.	N.D.
RP 4	3.58	N.D.	N.D.	N.D.	N.D.
RP 5	1.41	N.D.	0.34	N.D.	N.D.
RP 6	2.76	N.D.	0.24	N.D.	N.D.
RP 7	6.11	0.53	0.47	0.22	N.D.
VP 1	N.D.	N.D.	N.D.	N.D.	N.D.
VP 2	N.D.	N.D.	N.D.	N.D.	0.40
VP 3	N.D.	N.D.	N.D.	N.D.	N.D.
VP 4	N.D.	N.D.	N.D.	N.D.	N.D.

N.D., not determined; RP, recycled paper; VP, virgin paper.

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Other derivatives studied here were not found in measurable levels from recycled and virgin paper materials. In the absence of detailed regulations on migration of BP from paper and board materials, the SML for BP in plastics (0.6 mg/kg) can be used as a presumptive standard but for information purposes. Because there is no specific European legislation for the migration limit of BP derivatives on paper and cardboard, there is lack of information in the literature about the migration level of BP derivatives from food packaging materials.

CONCLUSION

The described method allowed a rapid multi-analyte quantification of BP and 17 other possible derivatives from virgin paper and recycled cardboard packaging for food applications by GC-MS. Good results regarding validation parameters were obtained with the method for BP quantification. So, the method can be successfully used to analyse these compounds in food packaging. This study can also be an important tool for the determination of BP migration from packaging materials to foods marketed in Turkey.

Seventeen of the BP derivatives were investigated all together for the first time in this paper. The results of the BP and other derivative determination in different types of paper and cardboard packaging materials showed that the higher content was always in the recycled packaging. More than half of the samples contained BP, four of the samples contained 4-methylbenzophenone, two of the samples contained 2,2-dimethoxy-2-phenylacetophenone and one of the samples contained 1-hydrocyclohexyl phenyl ketone. The result of the study showed that seven (63%) of the 11 food packaging materials tested exceeded the SML of 0.6 mg/kg. In each category of different recycled packaging, it was possible to observe many differences in the BP content.

This survey indicates that, generally, a high concentration of one PI and traces of other possible substitutes can be found in paper and cardboard packaging. These traces might also exist as residual levels of formerly used printing inks remaining on recycled paperboards. The ability of the PI (used in the printing process) to migrate through the vapour phase should be considered in a risk evaluation together with the right choice of a primary packaging to assure the correct protection in packaged food. It is the responsibility of the total production line, including the food industry and suppliers, to select the correct PI.

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