Analysis of Benzophenone and 4-Hydroxybenzophenone in Breakfast Cereal

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Key Words
Benzophenones, food packaging, chemical migrant, QuEChERS

Abstract
The use of QuEChERS dispersive SPE as a simple, fast, and quantitative sample preparation method for the GC-MS analysis of benzophenone and 4-hydroxybenzophenone in breakfast cereal has been demonstrated. Additionally, the suitability of the Thermo Scientific™ TraceGOLD™ TG-17MS GC column for benzophenones analyses has been shown. The average recoveries for the spiked benzophenone and 4-hydroxybenzophenone in breakfast cereal at 0.6 mg/kg were 101.7% and 82.3%, with relative standard deviations of 2.3% and 4.6% respectively, using the modified QuEChERS method described in EN15662.

Introduction

The Food Standard Agency (FSA) published a method for analyzing benzophenone and 4-hydroxybenzophenone from food[1]. Benzophenone is a chemical migrant, associated with inks and coating in food packaging that can migrate to the surface of the food. Directive 2002/72/EC has a specific migration limit (SML) for benzophenone at 0.6 mg/kg.

The FSA method uses a liquid-liquid extraction method that involves shaking the homogenized food matrix overnight with 1:1 acetonitrile/dichloromethane and then extracting the samples with the same mixture of solvents followed by centrifugation. The organic solvent is removed and the solid is re-extracted with 1:1 acetonitrile/dichloromethane. The combined organic extract is then back extracted with hexane to remove any potential fat from the matrix. Benzophenone is soluble in hexane and this can give reduced extraction recoveries. This method is highly laborious and can take up to 18 hours to perform the analysis.

Alternatively, a novel approach can be used to extract benzophenones from foods using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. QuEChERS is a dispersive solid phase extraction (SPE) technique initially developed for extracting multi-residue pesticides from fruits and vegetables. The extraction method can be applied for removing matrix from non-pesticide analytes such as benzophenones. The advantages of this methodology are speed, ease of execution, minimal solvent requirement, and cost. The QuEChERS methodology was developed by Anastassiades *et al.* [2] and has become widely used in food safety analyses.

The method is:

- Quick – provides high sample throughput. Typically eight samples can be prepared in under 60 min.
- Easy – requires less handling of extracts than other techniques, i.e. fewer steps are required.
- Cheap – needs less sorbent material and less time to process samples compared to other techniques.
- Effective – gives high and accurate recovery levels for a range of different compound types.
- Rugged – detects a large number of compounds, including non-polar and polar compounds.
- Safe – does not require the use any chlorinated solvents, unlike other techniques. Extraction is typically carried out using acetonitrile, which is both GC and LC compatible.

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A modified sample preparation approach, described in the European EN15662 QuEChERS procedure[3], was used for extracting benzophenones from breakfast cereal. This is a two-stage process: sample extraction, followed by dispersive SPE (Figure 1).

In the sample extraction stage, the food sample is homogenized to increase the available surface area of the sample to provide optimal extraction efficiency. The homogenized sample must be at least 80% hydrated for the extraction to work. The food sample is placed in the extraction tube with acetonitrile and a salt mix is then added comprising of magnesium sulfate, sodium chloride, sodium citrate tribasic dihydrate, and sodium citrate dibasic sesquihydrate [P/N 60105-337]. Magnesium sulfate ensures that upon addition of acetonitrile, a phase separation is induced between the water and organic solvent with the analytes of interest being extracted into the organic phase. The tube is then capped, shaken vigorously, and centrifuged.

The second stage of the QuEChERS method uses dispersive SPE, which involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents for removal of unwanted sample components. The sorbent combination of magnesium sulfate, PSA (primary and secondary amines) silica, and C18 [P/N 60105-225] in the sample clean-up tube removes fat from the matrix, reduces matrix effects, and therefore improves method robustness for extraction recoveries.

This fast and simple approach was taken to analyze and extract benzophenone and 4-hydroxybenzophenone from breakfast cereal. The method typically takes approximately 2 hours to complete, with reduced solvent usage compared to the liquid-liquid extraction method described above in the official method[1]. Six extractions of 0.6 mg/kg spiked level were used for the recovery experiments.

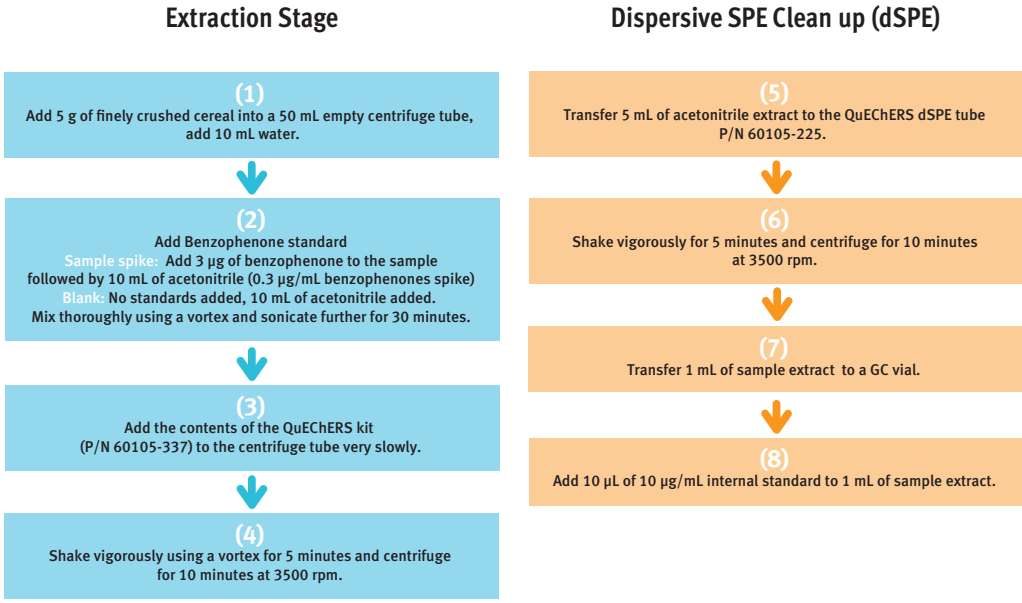

Figure 1: Sample preparation illustrating QuEChERS protocol

Experimental Details

Consumables		Part Number
Column:	TraceGOLD TG-17MS , 30 m x 0.25 mm x 0.25 µm	26089-1420
Septum:	BTO, 17 mm	31303211
Liner:	Splitless FocusLiner™ for 50 mm needle, 5 x 8 x 105 mm	453T2999
Column ferrules:	100% graphite ferrules for Thermo Scientific TRACE™ injector 0.1-0.25 mm ID	29053488
Column ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm ID	29033496
Injection syringe:	10 µL fixed needle syringe for Thermo Scientific TriPlus™ RSH Autosampler	365D0291
Vials and closures:	9 mm Wide Opening Screw Thread Vial Convenience Kit, 2 mL Clear Vial with Patch, Blue Polypropylene Closure with Clear PTFE/Blue Silicone Septa	60180-599

Consumables	Part Number
Fisher Scientific™ LC-MS Grade acetonitrile	A/0638/17
Fisher Scientific LC-MS Grade water	W/0112/17
Thermo Scientific HyperSep™ Dispersive SPE multipacks, 6000 mg magnesium sulfate, 1500 mg sodium chloride, 1500 mg sodium citrate dihydrate, 750 mg disodium citrate sesquihydrate. Each pack contains 50 metalized pouches with 50 empty centrifuge tubes and plug seal caps	60105-337
Thermo Scientific HyperSep Dispersive SPE clean-up product: 15 mL tube containing 1200 mg magnesium sulfate, 400 mg PSA, 400 mg C18	60105-225

Preparation of Calibration Standards

The calibration curve was constructed with benzophenone and 4-hydroxybenzophenone at the following concentrations: 50, 100, 300, 500, and 1000 µg/mL. To each 1 mL of calibration standard solution, 10 µL of 10 µg/mL benzophenone-d10 and 4-fluoro-4'-hydroxybenzophenone (internal standards) were added.

Separation Conditions

Instrumentation:	Thermo Scientific TRACE GC Ultra™
Carrier gas:	Helium
Column flow:	1.2 mL/min, Constant flow
Oven temperature:	80 °C (1.0 min), 30 °C/min, 280 °C (5 min)
Injector type:	Split/Splitless
Injector mode:	Splitless (1 min), 50 mL/min split flow
Injector temperature:	250 °C

Detector type:	Thermo Scientific ISQ™ Mass Spectrometer
Transfer line temperature:	280 °C
Source temperature:	250 °C
Ionization conditions:	EI
Electron energy:	70 eV

Scan window start time (min)	Compound name	Mass list (Quan ion), Qual ions	Dwell time (sec)
4.00	Benzophenone	(182), 77, 105	0.05
4.00	Benzophenone-d10 (IS)	(192), 110	0.05
7.50	4-hydroxybenzophenone	(198), 77, 105	0.05
7.50	4-fluoro-4'-hydroxybenzophenone (IS)	(216), 121	0.05

Table 1: SIM scan Parameters

Injection Conditions

Instrumentation:	Thermo Scientific TriPlus RSH Autosampler
Injection volume:	1 µL
Pre- and post-injection dwell time:	5 seconds

Results

To assess the method linearity, a calibration curve was constructed for benzophenone and 4-hydroxybenzophenone in acetonitrile using benzophenone-d10 and 4-fluoro-4'-hydroxybenzophenone as the internal standards (IS). The coefficients of determination (R^2) between the area ratio of sample and internal standard for both compounds were >0.999 (Figure 2, Table 2), demonstrating excellent method linearity using the Thermo Scientific TraceGOLD TG-17MS GC column.

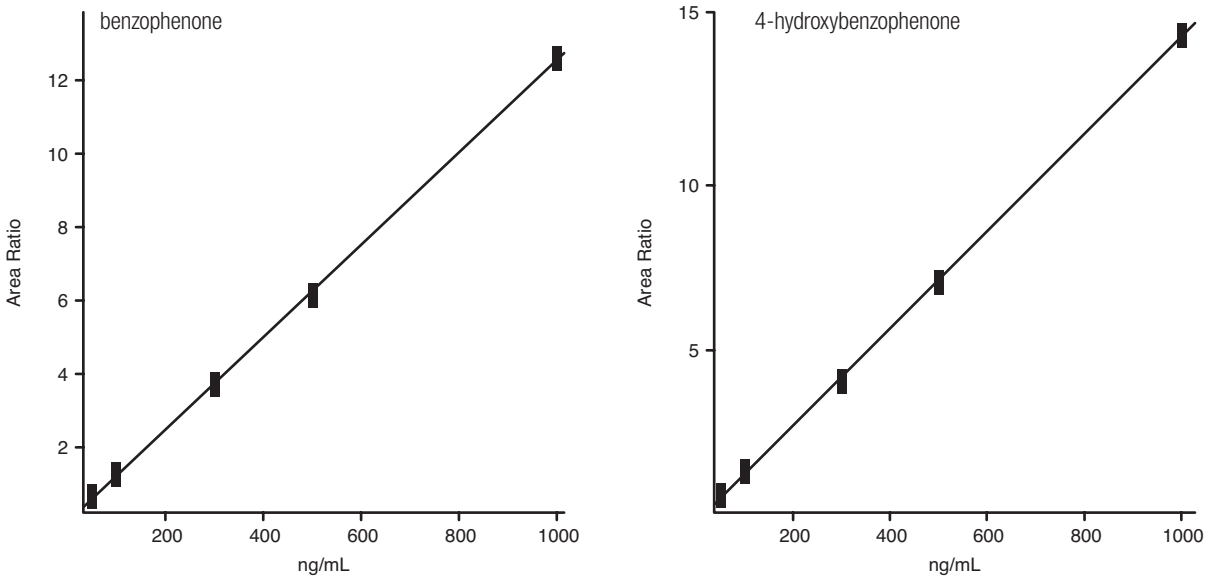

Figure 2: Calibration curve for benzophenone and 4-hydroxybenzophenone

Peak	Compound	t_R (min)	Linearity	% Recovery	%RSD (n=6)
1	Benzophenone-d10 (IS)	6.64	–	–	–
2	Benzophenone	6.67	0.9996	101.7	2.3
3	4-fluoro-4'-hydroxybenzophenone (IS)	8.13	–	–	–
4	4-hydroxybenzophenone	8.37	0.9996	82.3	4.6
a	Matrix impurities	6.79, 7.47	–	–	–

Table 2: Peak identification, linearity, and recovery data for benzophenone and 4-hydroxybenzophenone

The analysis was performed in SIM mode. Figure 3 shows the SIM chromatogram of spiked benzophenone and 4-hydroxybenzophenone in cereal matrix at 0.6 mg/kg. Six extractions of spiked samples were carried out and recoveries measured. The recoveries for the spiked benzophenone and 4-hydroxybenzophenone were 101.7% and 82.3%, with an average relative standard deviation (RSD) of 2.3% and 4.6%, respectively (Table 2).

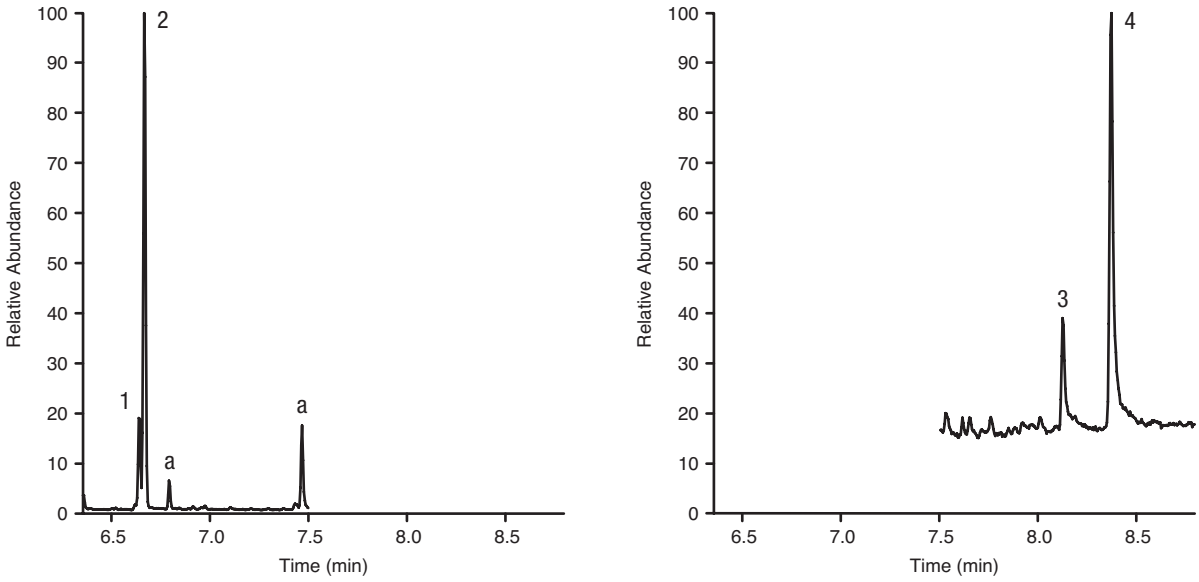

Figure 3: SIM chromatogram for benzophenone and 4-hydroxybenzophenone spiked at 0.6 mg/kg in breakfast cereal

Conclusion

The QuEChERS sample preparation method provided a fast and simple approach for extracting and analyzing benzophenone and 4-hydroxybenzophenone in breakfast cereal, achieving high recoveries and good reproducibility. The QuEChERS – GC-MS method was found to be linear in the concentration range of 50 to 1000 ng/mL. The TraceGOLD TG-17MS GC column provided a good chromatographic separation for all analytes studied.

References

1. Food Standards Agency: Survey of benzophenone and 4-hydroxybenzophenone migration from food packaging into foodstuffs. Number 18/06, November 2006. http://www.food.gov.uk/science/surveillance

2. M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, J AOAC Int 86 (2003) 412.

3. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE–QuEChERS method. European Standard EN15662:2008

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