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Simultaneous Determination of Antioxidants and Ultraviolet Stabilizers in Polypropylene Food Packaging and Food Simulants by High-Performance Liquid Chromatography

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Summary. The use of polypropylene materials in industry for food packaging is increasing. The presence of additives in the polymer matrix enables the modification or improvement of the properties and performance of the polymer, but these additives are potential risk for human health. In this context, an efficient analytical method for the quantitative determination of three antioxidants (2,6-di-tert-butyl-4-methylphenol (BHT), dibutylhydroxyphenylpropionic acid stearyl ester (Irganox 1076), and tns-(2.4-di-tert-butyl)-phosphite (Irgafos 168)) and five ultraviolet stabilizers (2-(2′-hydroxy-5′-methylphenyl) (UV-P), (2′-hydroxy-3′-tert-5′-methylphenyl)-5-chloroben zotriazole (UV-326), 2-(2′-hydroxy-3′,5′-di-tert-butylphenyl)-5-chlorobenzotriazole (UV-327), 2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol(UV-329), and 2-hydroxy-4(octyloxy) benzophenone (UV-531)) in polypropylene food packaging and food simulants by high-performance liquid chromatography (HPLC) has been developed. Parameters affecting the efficiency in the process such as extraction and chromatographic condition were studied in order to determine operating conditions. The analytical method showed good linearity, presenting correlation coefficients ($R \geq 0.9977$) for all additives. The limits of detection and quantification were between 0.03 and 0.30 μg mL^{-1} and between 0.10 and 1.00 μg mL^{-1} for eight analytes, respectively. Average spiked recoveries in blank polypropylene packaging and food simulants were in the range of 80.4–99.5% and 75.2–106.7%, with relative standard deviations in the range of 0.9–9.1% and 0.2–9.8%. Dissolving the polypropylene food packaging with toluene and precipitating by methanol was demonstrated more effective than ultrasonic extract with acetonitrile or dichloromethane for extracting the additives. The method was successfully applied to commercial polypropylene packaging determination, Irgafos 168 and UV-P were frequently found in six commercial polypropylene films, and the content ranged from 166.47 ± 5.11 to 845.27 ± 29.31 μg g^{-1} and 2.10 ± 0.29 to 19.23 ± 1.26 μg g^{-1}, respectively.

Key Words: food packaging, polypropylene, food simulant, antioxidants, ultraviolet stabilizers, chromatography

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Introduction

In recent years, plastics are widely used in food contact materials. Among the plastic polymers, polypropylene (PP) is used in many applications because of several advantages including its being easily shaped, unbreakable, solid, lightweight, recyclable, and transformable. All of these remarkable characteristics are partly due to the incorporation of various kinds of additives to the polymer matrix. These compounds enhance process ability and improve the polymer properties both during processing and under the conditions of use. As a result of their various applications, there have been some concerns about the migration of additives when the polymer packaging is in contact with food [1, 2]. The release of additives, initially present in the packaging, into product can cause undesirable flavors in the food or even promote toxicity and damage humans. Therefore, the determination of additives content in polymers is an important component of the safety assessment of food packaging materials, and the quantification and specific migration levels of these additives are also very important for the quality control of food.

Among a wide variety of polymer additives, antioxidants and ultraviolet stabilizers are commonly used in polypropylene packaging material. Antioxidants are both natural and synthetic compounds able to scavenge free radical and inhabit oxidation process, synthetic antioxidants are key ingredients in the compounding of polypropylene due to the limited stability of polyolefins to high temperature and ultraviolet (UV) light, and used in plastic to prevent the aging and keep the stability of plastic [3]. Ultraviolet stabilizers are used to slow down the degradation process by preferentially absorbing harmful UV radiation and dissipating it as thermal energy [4].

Nowadays, different extraction techniques were used to determine the level of additives in polymers, including conventional extraction techniques, namely, dissolution and precipitation of the polymer, soxhlet extraction, reflux and heating extraction, and new development extraction techniques such as microwave-assisted extraction, ultrasonic energy, supercritical fluid extraction, or accelerated solvent extraction [5]. Chromatographic techniques including high-performance liquid chromatography (HPLC) [6–8], gas chromatography (GC) [9, 10], liquid chromatography–mass spectrometry (LC–MS) [11, 12], and gas chromatography–mass spectrometry (GC–MS) [13, 14] were used as analytical methods. Meanwhile, different experimental conditions and procedures have also been employed to study specific migration levels of additives into food simulants [15–19]. However, to the best

of our knowledge, few methods investigated all food simulants according to Commission Regulation (EU) No. 10/2011 [20]. At the same time, it is generally known that a large variety of antioxidants and ultraviolet stabilizers are used in plastic. The lack of an efficient method for detecting more antioxidants and ultraviolet stabilizers in food packaging and food simulant or foodstuff is a problem that should be solved quickly.

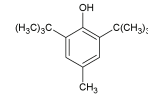
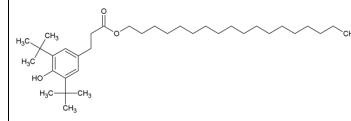
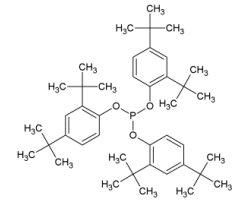
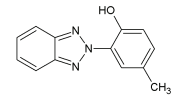
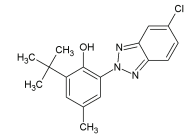
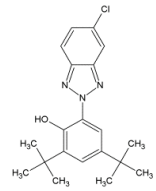
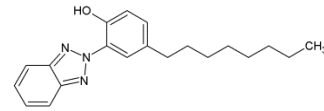
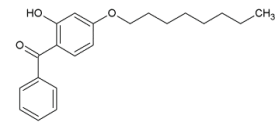
In this paper, the commonly used antioxidants (BHT, Irganox 1076, and Irgafos 168) and ultraviolet stabilizers (UV-P, UV-326, UV-327, UV-329, and UV-531) were studied. According to Commission Regulation (EU) No. 10/2011, 10% (*v*/*v*) ethanol, 3% (*w*/*v*) acetic acid, 20% (*v*/*v*) ethanol, 50% (*v*/*v*) ethanol, and substitute fatty food simulant isooctane were used. The aim of this study is to develop an efficient analytical method with suitable detection limits, good repeatability, and accuracy for the determination of antioxidants and ultraviolet stabilizers in polypropylene packaging and different food simulants to deal with the increasing demands on food packaging material safety.

Experimental

Chemicals and Reagents

The sources and some information of the model additives are presented in *Table 1*. Methanol of HPLC grade used as mobile phase in the HPLC was purchased from Merck (Darmstadt, Germany). Dichloromethane (analytical reagent grade), acetonitrile (HPLC grade), and toluene (analytical reagent grade) which were used for extraction solvents were obtained from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). For the preparation of the food-simulating solutions, acetic acid of analytical reagent grade (Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China) was used together with ethanol and isooctane of HPLC grade from TEDIA (Cincinnati, Ohio, USA). Other reagents used were sodium chloride (NaCl) and *n*-hexane of analytical reagent grade from Kermel Chemical Reagent (Tianjin, China). High-purity deionized water (resistivity 18.2 MΩ cm^{-1}) was prepared by using a water purification machine (Eped, Nanjing, China).

Table I. Detailed information of the studied additives

Abbreviation	Molecular weight	CAS no.	SML ($\mu g\ g^{-1}$)	Source	Structure
BHT	220.36	128-37-0	3.0	Fluka Cheme GmbH, Buchs Sigma-Aldrich (Augsburg, Germany)	
Irganox 1076	530.86	2082-79-3	6.0	Pure Chemical Analysis Co., Ltd. (Boenew, Belgium)	
Irgafos 168	646.94	31570-04-4	–	Sigma-Aldrich (Steinheim, Germany)	
UV-P	225.25	2440-22-4	30.0	Sigma-Aldrich (Steinheim, Germany)	
UV-326	315.80	3896-11-5	30.0	AccuStandard, Inc. (New Haven, USA)	
UV-327	357.88	3864-99-1	–	AccuStandard, Inc. (New Haven, USA)	
UV-329	323.43	3147-75-9	-	AccuStandard, Inc. (New Haven, USA)	
UV-531	326.43	1843-05-6	6.0	AccuStandard, Inc. (New Haven, USA)	

SML: specific migration limit allowed by Commission Regulation (EU) No. 10/2011.
"–" No restriction references in Commission Regulation (EU) No. 10/2011.

Instrumentation

The HPLC system was a Waters Alliance 2695 (Waters, Milford, MA, USA) equipped with a gradient pump, an automatic injector and a model 2996 photodiode array detector (PDA). LABUY-10LHT ultrasonic system (Hangzhou Labuy Instrument Co., Ltd., Hangzhou, China) was used for the sample pretreatment. TurboVap® II evaporation system (Caliper Life Sciences Inc., Hopkinton, USA) and QL-866 Vortex shaker (Haimen Qilinbeier Instrument Manufacturing Co., Ltd., Jiangsu, China) were used for sample concentration and homogenizing, respectively. Eppendorf Centrifuge 5801 R (Hamburg, Germany) and GZX-9420 MBE electric blast drying oven (Boxun Medical Equipment Industry Co., Ltd., Shanghai, China) were also used.

PP Samples

In order to verify the accuracy and precision of the proposed method, experimental PP films incorporated additives were obtained by double extrusion. Three different kinds of polypropylene pellets (PP-H, PP-B, and PP-R) were provided by China Petroleum and Chemical Corporation (Shanghai, China). Additives were mixed with virgin PP pellets and manufactured into particle by a twin-screw extrusion granulation line (Guangzhou PuTong Machinery and Equipment Manufacturing Co., Ltd., Guangzhou, China). The operating temperature for the nine zones of the screw was 180–190–195–200–200–200–200–200–195 °C; the die was at 200 °C. The screw speed ranged from 10 to 200 rpm. The pellets incorporated additives were passed through the double screw extruder twice to homogenize the mixture thoroughly. The second extrusion involved a single-screw extruder of 20 mm diameter with a barrel with a length-to-diameter ratio of 20 (Guangzhou Jin Fangyuan Machinery and Equipment Manufacturing Co., Ltd., Guangzhou, China). The temperature profile of the zones of the screw was 180–190–195–195 °C. It was used to manufacture films. The blank PP films containing no additives were obtained under similar conditions and used as controls. The thickness of samples was measured at five points spread out over the sample using a DRK 203B digital thickness meter (Derek Instrument Co., Ltd., Jinan, China), and the mean value of the samples was in the range of 0.047–0.051 mm. The densities (calculated from sample weight, thickness, and area) were in the range of 0.93–1.03 g cm^{-3} with no significant difference between the polymer types.

Six kinds of commercial PP films used for food packaging were selected in order to demonstrate the applicability of the method for the analysis of real matrix samples. These samples were supplied by Shenzhen Border Inspection and Quarantine Bureau. They were numbered from sample 1 to sample 6. The information of these samples is presented in *Table II*.

Table II. The information of commercial polypropylene packaging materials

Sample	Type of material[a]	Thickness ± SD (mm) (n = 6)	Density ± SD (g cm^{-3}) (n = 6)	Remark	Food packaged
1	PP	0.059 ± 0.003	0.99 ± 0.05	Printed, shaped	Bread crumbs
2	BOPP	0.024 ± 0.001	0.96 ± 0.04	Unprinted, unshaped	Cake
3	PP	0.075 ± 0.003	1.06 ± 0.01	Printed, shaped	Coconut soft flour cake
4	OPP	0.078 ± 0.001	1.01 ± 0.01	Printed, shaped	Scallops noodle
5	OPP/CPP	0.077 ± 0.002	1.00 ± 0.02	Printed, shaped	Shrimp noodle
6	OPP/CPP	0.069 ± 0.002	1.02 ± 0.02	Printed, shaped	Egg noodle

[a]PP, polypropylene; BOPP, biaxial oriented polypropylene; OPP, oriented polypropylene; OPP/CPP, oriented polypropylene and cast polypropylene composite film.

Preparation of Standard Solutions and Food Simulants

Primary stock solutions of BHT and UV-531 were prepared with analytical accuracy dissolving 10 mg standard in 10 mL methanol (1000 μg mL^{-1}). For Irganox 1076, Irgafos 168, UV-P, UV-326, UV-327, and UV-329, stock solution (1000 μg mL^{-1}) was prepared with analytical accuracy, dissolving 10 mg standard in 1.0 mL dichloromethane, and then diluted to 10 mL with methanol. Mixture stock solution at a concentration of 100 μg mL^{-1} was obtained by mixing 1.0 mL of each kind of the previously mentioned individual stock solution and then diluting to 10 mL with methanol. Subsequent dilutions were prepared also in methanol in the range of 0.1–50.0 μg mL^{-1} for the HPLC calibration curves. All solutions were stored in amber bottles at 4 °C in the refrigerator.

One hundred, two hundred, or five hundred milliliters of absolute ethanol was placed into a volumetric flask and made up to 1000 mL with deionized water to give 10% (*v*/*v*) ethanol, 20% (*v*/*v*) ethanol, and 50% (*v*/*v*) ethanol, respectively. Thirty grams of acetic acid was dissolved in 1000 mL deionized water to give 3% (*w*/*v*) acetic acid solution.

HPLC Analysis

The analysis of standards and extracts was made on a Waters Alliance 2695 HPLC system. The signal acquired from detector was recorded by a personal computer operated under the Empower 2 chromatographic software (Waters, Milford, MA, USA). Chromatographic separation was performed with an XBridge™ C_{18} column (250 mm × 4.6 mm, 5 μm) analytical column at 30 °C. A gradient elution method with methanol–water binary mixture was used. The initial proportion was 80% methanol–20% water (*v*/*v*); linear gradient was up to 100% methanol at 15 min, held for 15 min, and returned to the initial condition in 5 min. The total run time of each analysis was 35 min. The wavelength setting on the PDA detector was 275 nm, the flow rate was 1.0 mL min^{-1}, and the injection volume was 20 μL. Each compound was identified by comparison of its retention time with corresponding peak in the standard solution and its UV spectrum.

PP Sample Preparation

Pure solvents or mixtures of acetonitrile, chloroform, isopropanol, cyclohexane, and dichloromethane represent the most commonly used solvents in polymer preparation [5]. In this work, acetonitrile and dichloromethane were compared to assess their extraction efficiency for additives from PP material. Toluene was also used to dissolve the PP film to evaluate the extraction efficiency for additives. PP film was rinsed using ultrapure water, rubbed with absorbent cotton, and dried before use, then cut into small pieces of 0.5 × 0.5 cm for further extraction.

(1) Ultrasonic extract with acetonitrile (or dichloromethane)

0.2 g PP sample was weighed, placed in a 100-mL conical flask with glass top, and contacted overnight with 10 mL acetonitrile (or dichloromethane) for maceration. Samples were mechanically shaken for 3 min and extracted

for 30 min in an ultrasonic system at 30 °C. After this time, the extract was removed and filtered through a 0.22-μm pore-size nylon membrane filter. The concentration of additives in PP sample was analyzed by HPLC–PDA. When dichloromethane was used as extraction solvent, the extract was removed and concentrated to near dryness at 40 °C by a TurboVap® II evaporation system, taken up in 2 mL of methanol, filtered through a 0.22-μm pore-size nylon membrane filter, and analyzed by HPLC. To ensure complete extraction of the polymer sample, the extraction process was repeated. Complete extraction was assumed to have taken place if the amount of additive found in the second extract was 5% or less of the first. If this was not the case, the samples were extracted a third time.

(2) Dissolved with toluene and precipitation by methanol

0.2 g PP sample was dissolved in 10 mL toluene, under heating and stirring. Continuously, 20 mL methanol was added in order to precipitate the polymer. The precipitate was then collected by vacuum filtration and used for a second cycle of dissolution. The solution from each cycle was collected and diluted to 80 mL with methanol, and an aliquot of the solution was filtered with a 0.22-μm pore-size nylon membrane filter; the concentration of additives was analyzed by HPLC.

During the ultrasonic extraction, aluminum foil was used to seal the conical flask to avoid solvent volatilization. The experiment was performed in triplicate. Blank PP sample without additives was used as reference, extracted, and analyzed under the same conditions.

Experiment to Determine Additives in Food Simulants

Commission Regulation (EU) No. 10/2011 proposed 10% (*v*/*v*) ethanol, 3% (*w*/*v*) acetic acid, 20% (*v*/*v*) ethanol, 50% (*v*/*v*) ethanol, and vegetable oil as food simulants for migration test. Thinking of the difficulty of working with vegetable oil in HPLC systems, isooctane was used as an alternative fatty food simulant.

The ethanol–water solution was directly injected into the HPLC instrument, while for the isooctane preparations, the sample was evaporated to dryness and redissolved with 2.0 mL methanol immediately before HPLC injection. A previous liquid–liquid extraction method was modified and applied to the extraction of additives from acetic acid–water solution [21]. Briefly, 10 mL of acetic acid–water simulant was removed in a test tube with

glass stopper (25 mL) and 5 mL *n*-hexane was added as extraction solvent; 0.5 g of NaCl was previously added to the test tube, and then, the test tube was shaken with orbital shaker. Samples were extracted for 30 min in an ultrasonic system at 30 °C and centrifuged for 5 min at 2500 rpm. Subsequently, organic phase was removed and extraction was repeated once; then, *n*-hexane extracts were combined and evaporated to near dryness and taken up in 2 mL of methanol. The concentration of additives in food simulants was analyzed by HPLC. The simulant of each sample was analyzed in triplicate. A blank prepared with only simulant was used as reference, exposed, and analyzed under the same conditions.

Method Validation

The presented method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, repeatability, and stability under the optimum conditions.

Linearity of HPLC for the Determination of Additives

To obtain the calibration curves for the analytes, mixed standard solutions ranging from 0.1 to 50.0 μg mL^{-1} were injected from low concentration to high concentration, and each solution was injected in triplicate with a volume of 20 μL. The quantification was based on the external calibration graph obtained by plotting the individual peak areas against the concentration of the calibration standards.

Limits of Detection and Limits of Quantitation

The LOD and LOQ for the studied additives were determined as the lowest concentration giving responses of three and ten times the average of the baseline noise. In this work, serially diluted standard solutions were injected from high concentration to low concentration until the response of every additive was three and ten times the average of the baseline noise, respectively.

Accuracy and Repeatability of the Method

In order to check the accuracy of the method, a recovery study was performed and it was tested by standard addition procedure. As precision of-

ten varies with analyte concentration and matrix type, the accuracy experiment of the method was performed for blank PP sample and different food simulants spiked with antioxidants and ultraviolet stabilizers at three levels, and three replicates (n = 3) were used for each concentration level. For blank PP sample, the experiment was performed for 0.2 g blank sample spiked with 20, 80, and 200 μg g^{-1} of each additive. The extraction procedure was followed as described in the extraction section (dissolved with toluene and precipitation by methanol). For food simulants, 10 mL simulant spiked at a concentration of 2, 5, and 10 μg mL^{-1} of each additive in glass tube was immediately shaken by vortex mixing for 3 min. The glass tube was then immersed in an electric blast drying oven and maintained at 70 °C for 2 h to simulate the worst case. The extraction procedure for food simulants was followed as described above. The recoveries were calculated as the ratio of the experimentally observed concentration to the theoretical concentration. The repeatability was expressed as the relative standard deviation (RSD) of the recovery values. Each analytical sequence was composed of solvent blank, calibration standard, reference blank sample (PP film or food simulant without additives), and sample (PP film or food simulant) which spiked the target additives.

Stability of the Studied Additives

Intra-day and inter-day precision were used to determine the stability of the additives. The intra-day precision was evaluated with three different concentration levels of mixed standard solutions under the optimized conditions three times within 1 day. Inter-day precision was assessed at the same three levels on 3 days. These two types of precisions were expressed as the RSD (%) of a series of measurement.

Alongside the intra-day and inter-day precision, in order to determine the potential loss of additives in food simulants during the heating procedure, a known amount of standard in 50% (v/v) ethanol and isooctane (2, 5, and 10 μg mL^{-1}) was performed; each spiked level was divided into four parts, three were heated for 2 h at 70 °C and then analyzed by HPLC, and one was analyzed directly. The areas of the heated samples were compared to the area for the unheated sample.

Purity Angle Test

The test was carried out using peak purity judgment, which is part of the Waters HPLC–PDA system for handling chromatographic data. The princi-

ple of peak purity judgment is to obtain purity threshold and purity angle of individual peaks. A component with a peak purity threshold higher than its purity angle would be considered most likely to be a pure substance; no coeluting component and the separation would be acceptable.

Statistical Analysis

Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) 16.0 (IBM SPSS Inc., New York, NY, USA) statistical software. $p < 0.05$ was identified as being statistically significant at the 95% confidence level.

Results and Discussion

Optimization of Separation Conditions

The chromatographic conditions were studied to obtain the best peak shape and an effective separation of all analytes with good stability of retention times. First, chromatographic conditions were built up as referred to the literature [22], the chromatographic condition was a mobile phase of methanol as eluent A and water as eluent B. Initial experiments were (1) 90% A increase to 95% A during 5 min, (2) increase to 100% A during 5 min, (3) 100% A kept for 15 min, and (4) decrease to 90% A during 5 min. The total time was 30 min. However, the eight additives did not show good resolution, the chromatographic peaks of UV-P and BHT are overlapped *(Fig. 1 (c))*. Thus, experiments were performed by testing different initial proportion for mobile phase (methanol–water = 80:20, 85:15, 95:5, 100:0) in order to obtain a good response. From the results of the test series, it was found that a proportion of A started at 80% or 100% was most effective resolution for UV-P and BHT *(Fig. 1 (a)* and *(e))*. However, when the initial proportion of A was set 100%, the separation of UV-326 and UV-327 was not excellent. Take the separation of eight additives into account, the initial proportion of methanol–water was set 80:20, and the optimum conditions reached are those described above.

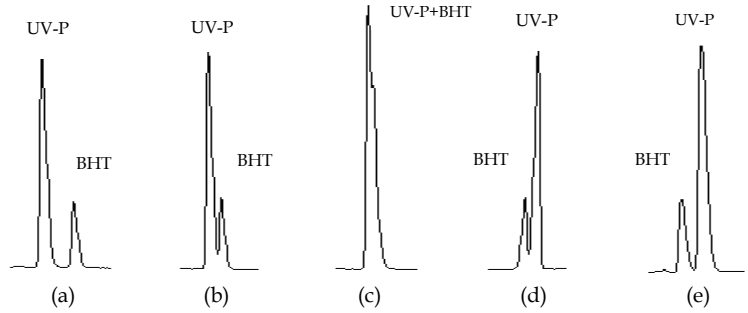


Fig. 1. Chromatographic peaks of UV-P and BHT in different initial proportion of mobile phase (methanol–water = 80:20 (a), 85:15 (b), 90:10 (c), 95:5 (d), and 100:0 (e))

Optimization of Extraction Condition for PP Film

Least significant difference (LSD) multiple comparison was used to compare the content of additives obtained by the different method at a 95% confidence level. The results of statistical analysis indicated that the extraction efficiency of dicloromethane was higher than acetonitrile for most additives. This could be explained by high affinity of dicloromethane to polypropylene, which causes swelling of the polymer and facilitates additives penetration. Additionally, there was a statistical difference in extraction efficiencies for ultrasonic extraction and dissolution–precipitation method. Unlike acetonitrile or dicloromethane, toluene can make polypropylene soften and further damage the structure of the polymer, which make the additive molecules escape easily from the constraint of polymer matrix; the inherently higher extraction efficiencies of toluene are supportive to our findings. Dissolving the PP material with toluene was demonstrated an effective way to promote the extraction of additives. This preparation method may not only limit to the additives in this study, and may be applied in routine analysis for other additives in polypropylene material.

Optimization of Extraction Condition for Food Simulants

Liquid–liquid extraction method was reported to extract additives from aqueous samples [21, 23]. In our view, any extra step may cause the potential loss of additives during the preparation. Consequently, the ethanol–water solution and acetic acid–water solution were directly injected into the HPLC instrument initially, while for the isooctane preparations, the

sample was evaporated to dryness and redissolved with 2.0 mL methanol immediately before HPLC injection. A volume of 10 mL of food simulant (10% (*v*/*v*) ethanol, 3% (*w*/*v*) acetic acid, 20% (*v*/*v*) ethanol, 50% (*v*/*v*) ethanol, and isooctane) spiked with the antioxidants and ultraviolet stabilizers were employed for all analyses. Good recoveries were achieved for additives in ethanol–water solution and isooctane, while the recoveries for additives in acetic acid–water solution were poor. This can be attributed to the low water solubilities for the studied additives. Thus, a modified liquid–liquid extraction method was performed. Ten milliliters 3% (*w*/*v*) acetic acid solution spiked at a concentration of 2, 5, or 10 μg mL^{-1} of each additive in glass tub4e, 5 mL of *n*-hexane and 0.5 g of NaCl are chosen, and sample was extracted twice. In order to facilitate observation, the recovery data of additives with spiked 10 μg mL^{-1} level was selected to plot. As can be seen in *Fig. 2*, recovery was significantly increased; the same has occurred at other two concentrations.

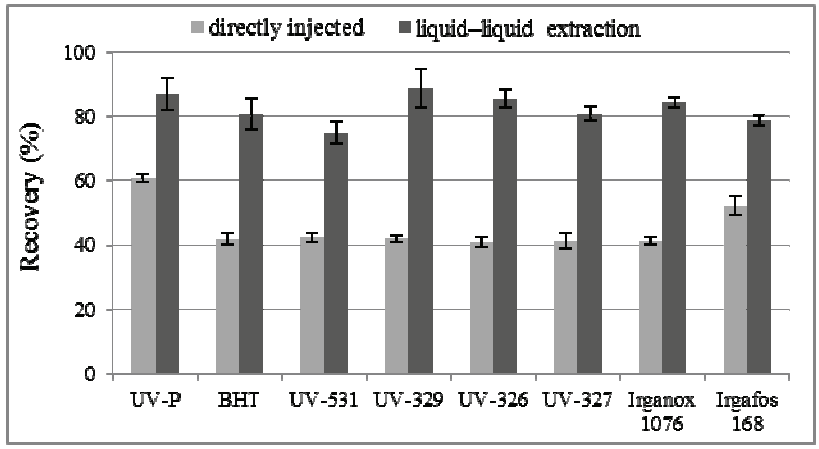


Fig. 2. Recoveries of additives in spiked 3% (*w*/*v*) acetic acid solution (10 μg mL^{-1}) using two methods ($n = 3$)

Method Validation

In this work, the studied additive responses were found to be linear over the concentration ranges selected with good correlation coefficients; the correlation coefficients (R) were greater than 0.99. The LODs and LOQs of eight additives were in the range of 0.03–0.30 μg mL^{-1} and 0.10–1.00 μg mL^{-1}. Detailed information regarding the calibration curves, linear 4ranges, LODs, and LOQs is listed in *Table III*.

Table III. Retention time, slope and intercept of linearity equation, linearity range, correlation coefficient, purity angle, purity threshold, LOD and LOQ of the target additives (n = 3)

Additive	Retention time ±SD (min)	Slope ± SD	Intercept ± SD	Linearity range (μg mL^{-1})	Correlation coefficient/R	Purity angle (purity threshold)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
UV-P	9.42 ± 0.05	32,005 ± 2356	2396 ± 139	0.1–50.0	0.9999	0.124 (0.311)	0.03	0.10
BHT	10.64 ± 0.01	8531 ± 594	1734 ± 457	0.5–50.0	0.9998	0.502 (0.724)	0.15	0.50
UV-531	15.56 ± 0.01	32,236 ± 259	1016 ± 83	0.1–50.0	0.9998	0.198 (0.319)	0.03	0.10
UV-329	16.39 ± 0.01	20,333 ± 275	978 ± 86	0.1–50.0	0.9998	0.215 (0.313)	0.03	0.10
UV-326	19.03 ± 0.01	13,941 ± 804	347 ± 75	0.5–50.0	0.9997	0.129 (0.311)	0.15	0.50
UV-327	20.18 ± 0.01	11,958 ± 854	951 ± 495	0.5–50.0	0.9999	0.152 (0.328)	0.15	0.50
Irganox 1076	26.00 ± 0.01	3343 ± 94	462 ± 153	1.0–50.0	0.9995	1.351 (1.655)	0.30	1.00
Irgafos 168	28.73 ± 0.05	4179 ± 138	694 ± 256	1.0–50.0	0.9977	0.500 (0.722)	0.30	1.00

The recoveries are presented in *Table IV*. The achieved recoveries of eight analytes in blank PP sample are between 80.4% and 99.5% with RSDs lower than 10% at concentration of 20, 80, and 200 μg g^{-1}. The recoveries of additives in food simulants are in the range of 75.2–106.7% at concentration of 2.0, 5.0, and 10.0 μg mL^{-1}, with RSDs between 0.2 and 9.8%. Since the excellent recoveries and repeatability were obtained within the framework of this study, the method is considered reliable for the determination of the target additives in polypropylene material and food simulants proposed by Commission Regulation (EU) No. 10/2011.

The intra-day precision and the inter-day precision are reported in *Table V*. For the intra-day precision, all of the RSDs were less than or equal to 3.5%. For the inter-day precision, the RSDs were below 11%. Nevertheless, the high value of the inter-day precision for several compounds indicates that the response of the instrument varies over time. In order to overcome this drawback and to obtain a reliable method, the calibration curves must be prepared and analyzed independently for each analysis batch.

Table IV. Recoveries and precision for spike the eight additives in blank PP and food simulants (n = 3)

	Fortified concentration	Recovery (%) (RSD (%))							
		UV-P	BHT	UV-531	UV-329	UV-326	UV-327	Irganox 1076	Irgafos 168
Blank PP	20 μg g^{-1}	98.4 (4.3)	81.6 (3.6)	91.6 (5.8)	81.8 (6.9)	80.4 (0.9)	82.4 (2.3)	85.9 (3.8)	81.3 (6.9)
	80 μg g^{-1}	99.5 (6.5)	83.1 (3.2)	95.7 (3.9)	83.9 (5.5)	80.7 (2.3)	83.0 (5.0)	82.2 (5.5)	87.2 (7.5)
	200 μg g^{-1}	88.0 (7.3)	85.1 (2.9)	83.0 (7.9)	83.4 (5.6)	88.8 (5.4)	85.9 (6.1)	82.1 (2.9)	92.5 (9.1)
10% (v/v) Ethanol	2.0 μg mL^{-1}	89.6 (0.6)	78.1 (4.9)	89.9 (0.8)	84.5 (2.6)	80.9 (0.3)	83.6 (1.4)	91.6 (2.8)	94.5 (0.5)
	5.0 μg mL^{-1}	93.3 (0.4)	75.6 (5.0)	95.4 (0.2)	95.1 (1.3)	92.1 (2.1)	95.9 (5.0)	87.9 (4.5)	100.2 (2.3)
	10.0 μg mL^{-1}	95.4 (0.2)	79.8 (3.7)	95.8 (0.4)	96.4 (0.6)	95.4 (1.9)	96.2 (2.9)	82.0 (3.7)	90.5 (5.3)
3% (w/v) Acetic acid	2.0 μg mL^{-1}	79.0 (4.4)	75.5 (9.6)	76.9 (5.6)	90.2 (7.2)	88.2 (8.1)	78.9 (9.1)	76.3 (8.9)	75.9 (2.5)
	5.0 μg mL^{-1}	82.8 (5.0)	82.0 (4.3)	77.6 (5.8)	83.7 (4.7)	81.6 (6.1)	75.2 (4.5)	76.4 (9.2)	77.2 (7.0)
	10.0 μg mL^{-1}	86.8 (5.8)	80.6 (5.7)	75.8 (4.5)	88.7 (6.8)	85.5 (3.3)	80.7 (2.7)	84.2 (2.0)	78.8 (2.0)
20% (v/v) Ethanol	2.0 μg mL^{-1}	89.9 (2.0)	89.4 (2.9)	84.5 (2.0)	80.7 (2.6)	86.9 (3.7)	89.3 (6.6)	92.9 (4.4)	100.4 (0.6)
	5.0 μg mL^{-1}	88.6 (0.5)	85.8 (9.8)	89.5 (1.5)	92.4 (0.7)	89.1 (1.8)	87.9 (4.3)	82.1 (6.1)	94.9 (2.4)
	10.0 μg mL^{-1}	95.1 (0.5)	85.0 (2.7)	93.9 (0.5)	97.7 (0.3)	94.1 (3.2)	96.4 (0.5)	89.7 (4.2)	90.8 (2.6)
50% (v/v) Ethanol	2.0 μg mL^{-1}	99.3 (2.2)	100.4 (1.8)	106.3 (2.2)	106.7 (4.1)	105.8 (4.2)	103.7 (2.1)	96.5 (3.9)	87.8 (0.9)
	5.0 μg mL^{-1}	96.3 (1.0)	93.0 (2.5)	95.0 (0.4)	96.6 (5.6)	94.0 (1.0)	95.1 (1.1)	80.6 (2.1)	99.6 (3.3)
	10.0 μg mL^{-1}	106.0 (0.4)	102.8 (0.8)	102.6 (3.1)	105.8 (3.2)	101.3 (5.1)	98.7 (1.2)	91.3 (9.3)	84.7 (7.7)
Isooctane	2.0 μg mL^{-1}	101.8 (3.5)	106.3 (4.2)	96.6 (3.3)	91.3 (6.7)	90.8 (3.3)	91.7 (8.2)	92.5 (2.3)	89.2 (0.8)
	5.0 μg mL^{-1}	102.4 (0.9)	93.5 (1.1)	94.1 (2.3)	96.7 (9.0)	90.8 (5.5)	93.5 (4.5)	81.4 (1.3)	106.1 (4.2)
	10.0 μg mL^{-1}	97.7 (5.8)	105.7 (4.1)	103.6 (5.2)	99.0 (6.0)	105.3 (1.8)	103.4 (1.8)	87.6 (9.4)	95.6 (9.6)

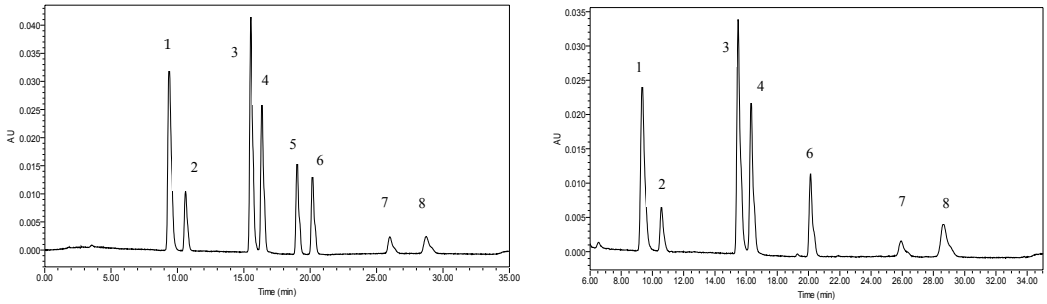
The areas of the heated samples were compared to the area for the unheated sample, showing good recoveries (larger than 91%) for all of the additives at the concentration of 2, 5, and 10 μg mL^{-1}. It means that there was no obvious loss of additives in the heating procedure. The areas of the three heated samples had relative standard deviations less than 3.6% for eight additives, showing good reproducibility in the heating procedure.

Table V. Intra- and inter-day stability of eight additives [intra-day/inter-day (n = 3)]

Concentration (μg mL^{-1})	RSD of concentration (%) intra-day/inter-day							
	UV-P	BHT	UV-531	UV-329	UV-326	UV-327	Irganox 1076	Irgafos 168
1.0	2.3/8.5	2.9/3.2	2.0/0.3	1.6/3.9	1.8/6.8	3.1/4.8	3.2/1.8	3.5/6.3
5.0	1.4/4.5	0.8/2.7	1.3/5.5	1.1/4.3	0.7/1.6	1.4/0.9	1.9/10.1	2.4/6.1
20.0	0.9/1.1	0.5/0.1	1.0/1.1	0.5/0.8	0.3/0.4	1.0/0.1	2.0/9.9	1.6/10.5

The purity threshold and purity angle values in the chromatogram of all the studied chemicals are mentioned in *Table III*, which illustrates that the peaks obtained high purity.

A typical chromatogram of the selected eight additives (20 μg mL^{-1} standard solution) is shown in *Fig. 3(a)*.



(a) (b)

Fig. 3. HPLC chromatogram of mixed standard solution at the concentration of 20 μg mL^{-1} (a) and extracted solution of experimental PP-H film (b) (1 UV-P, 2 BHT, 3 UV-531, 4 UV-329, 5 UV-326, 6 UV-327, 7 Irganox 1076, and 8 Irgafos 168)

Additive Content in Experimental PP Films

The content of additives in three experimental PP films (PP-H, PP-B, and PP-R), as discussed in the previous section, was determined by ultrasonic extraction and dissolution–precipitation as described in the extraction section. *Table VI* shows the results. It was observed that there was no UV-326 in three experimental PP films using different extraction methods. It is likely that UV-326 precipitated during the pelletizing and extrusion process for its low solubility and not added in the PP film. It agrees with the reports of Monteiro [24] and Nerín [25] which proved that UV-326 is not soluble and difficult to incorporate into polymer. The HPLC chromatogram of extracted solution of PP-H film (with additives) is shown in *Fig. 3(b)*.

Table VI. Content of additives achieved in three experimental PP films using different preparation methods [content ± SD (μg g^{-1}), n = 3]

Additive	PP-H			PP-B			PP-R		
	Ultrasonic extraction		Dissolution + precipitation	Ultrasonic extraction		Dissolution + precipitation	Ultrasonic extraction		Dissolution + precipitation
	Acetonitrile	Dichloro-methane		Acetonitrile	Dichloro-methane		Acetonitrile	Dichloro-methane	
UV-P	5962 ± 292^{a}	3957 ± 113^{b}	6048 ± 151^{a}	6712 ± 130^{a}	3875 ± 85^{b}	6954 ± 400^{a}	5844 ± 341^{a}	4861 ± 288^{a}	6162 ± 427^{a}
BHT	3386 ± 175^{a}	3239 ± 354^{a}	5404 ± 69^{b}	3997 ± 93^{a}	2420 ± 223^{b}	5558 ± 404^{c}	2630 ± 60^{a}	3451 ± 352^{a,b}	4998 ± 393^{b}
UV-531	6335 ± 399^{a}	4947 ± 401^{b}	7202 ± 142^{c}	7458 ± 190^{a}	4383 ± 182^{b}	8041 ± 503^{a}	5711 ± 275^{a}	5816 ± 515^{a,b}	7045 ± 458^{b}
UV-329	2901 ± 137^{a}	4859 ± 361^{b}	7234 ± 145^{c}	3999 ± 156^{a}	4211 ± 110^{a}	8113 ± 602^{b}	2333 ± 107^{a}	5589 ± 500^{b}	6888 ± 513^{b}
UV-326	–	–	–	–	–	–	–	–	–
UV-327	2101 ± 109^{a}	4518 ± 251^{b}	7242 ± 115^{c}	2881 ± 179^{a}	3989 ± 105^{b}	7992 ± 531^{c}	1715 ± 117^{a}	5506 ± 470^{b}	7135 ± 475^{b}
Irganox 1076	2305 ± 239^{a}	4338 ± 206^{b}	6381 ± 276^{c}	4697 ± 185^{a}	3930 ± 189^{b}	6903 ± 548^{c}	2166 ± 222^{a}	5227 ± 385^{b}	6142 ± 133^{b}
Irgafos 168	1969 ± 116^{a}	4123 ± 110^{b}	6607 ± 377^{c}	2617 ± 174^{a}	4637 ± 413^{b}	7226 ± 130^{c}	1839 ± 88^{a}	5073 ± 592^{b}	7060 ± 606^{c}

For each additive, different letters (a, b, c) indicate significant differences at 95% confidence level.

Analytical Applications

Six commercial PP films used for food packaging were prepared and determined using the obtained analytical method. The quantitative values of the analyses in different samples are shown in *Table VII*. It can be seen that

Irgafos 168 was found in all samples and the content ranged from 166.47 ± 5.11 to 845.27 ± 29.31 μg g^{-1}; UV-P was frequently found in samples 1–5, and the content ranged from 2.10 ± 0.29 to 19.23 ± 1.26 μg g^{-1}. Additionally, sample 1 contained UV-531, with a content of 5.08 ± 0.85 μg g^{-1}, and UV-329 was only found in sample 4, having a content of 12.71 ± 0.53 μg g^{-1}.

Table VII. Content of additives in studied commercial packages (n = 3)

Additive	Content ± SD (μg g^{-1})					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
UV-P	2.10 ± 0.29	2.33 ± 0.31	2.67 ± 0.14	19.23 ± 1.26	10.74 ± 0.18	nd
BHT	nd	nd	nd	nd	nd	nd
UV-531	5.08 ± 0.85	nd	nd	nd	nd	nd
UV-329	nd	nd	nd	12.71 ± 0.53	nd	nd
UV-326	nd	nd	nd	nd	nd	nd
UV-327	nd	nd	nd	nd	nd	nd
Irganox 1076	nd	nd	nd	nd	nd	nd
Irgafos 168	166.47 ± 5.11	845.27 ± 29.31	352.50 ± 2.20	272.16 ± 29.97	367.45 ± 36.26	431.51 ± 28.31

Conclusions

In this paper, a quantitative method for the determination of antioxidants and ultraviolet stabilizers in polypropylene food packaging and five food simulants has been developed. Dissolving the PP sample with toluene and precipitating by methanol was demonstrated an effective way to promote the extraction of additives. The method showed good linearity, accuracy, precision, and repeatability. Six commercial PP films used for food packaging were determined; Irgafos 168 and UV-P were frequently found in the selected polypropylene packaging. It is valuable in real application. Moreover, based on this work, migration test will be set up to determine the effect of different experimental conditions between polymer packaging and food simulants/foodstuff, such as contact time, temperature, light intensity and quality, and so on, and find the migration regularity of antioxidants and ultraviolet stabilizers

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