Determination of Irganox compounds extracted from food packaging using 4 food simulants

Quantification of Irganox chemicals using the QTRAP 6500+ system

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Introduction

This technical note demonstrates the accurate and precise quantification of 16 Irganox compounds spiked into food simulants and in a plastic food packaging extract. Most Irganox compounds met the acceptable accuracy (70-130%) and precision criteria (%CV <15%) when quantifying from a single solvent external calibration curve, eliminating the need for matrix-matched standards. The lowest calibration standard measured ranged from 0.025 to 0.50 ng/mL, demonstrating the sensitivity of the QTRAP 6500+ system.

Human health can be impacted by chemicals that migrate into foods and beverages from food contact materials (FCMs), such as wrappers and containers. Understanding the risk of FCM chemicals requires sensitive and accurate analytical methods. Extractables are chemicals that are released when the FCMs are stressed, such as using solvents, elevated temperatures, solvent exposure time and agitation. Conversely, leachables are chemicals that migrate under ambient conditions.

The method presented in this technical note focuses on extractables found in food packaging. The method was developed for the quantification of a diverse suite of 16 Irganox compounds found in food packaging using 4 food simulants, as outlined by the EU regulations.[1]

Key features of the QTRAP 6500+ system for the analysis of Irganox compounds

- Two external calibration curve preparations that were dependent on compound-specific solubility were used to analyze food packaging samples in 4 different food simulants, based on EU regulations
- The sensitivity of the QTRAP 6500+ system enabled accurate quantification below the 10 ng/mL EU regulatory level
- Linearity was observed between 0.025 and 50 ng/mL with a r^2 value >0.99
- Post-spike sample accuracy was observed between 70% and 130% with %RSD <15% when quantified using an external calibration curve (n=6)

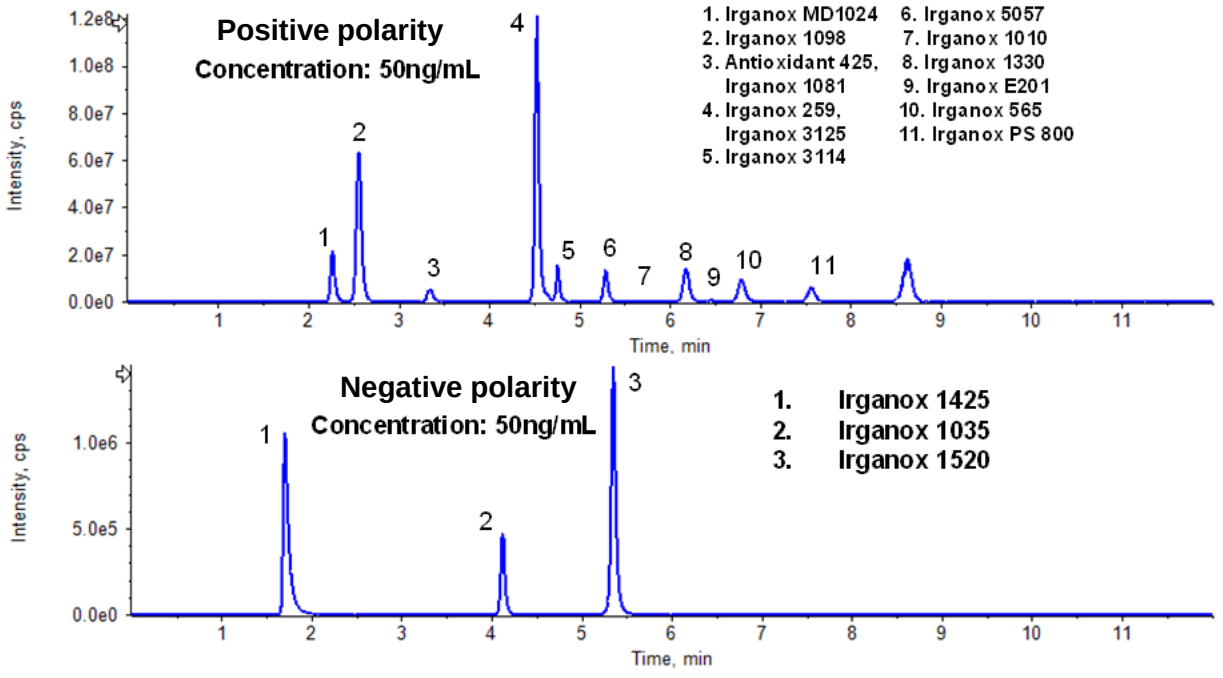

Figure 1. Overlaid extracted ion chromatograms (XICs) of 16 Irganox compounds. Positive ion (top panel) and negative ion (bottom panel) analytes were acquired in electron spray ionization (ESI) mode and are shown for a 50 ng/mL standard.

Methods

Standard preparation: All standards were weighed to approximately 1 mg, dissolved in 1 mL of methanol and sonicated for 5 min. Irganox 1529 (n-hexane) and Irganox E201 (ethanol) were available as neat standard solutions. A 50 µg/mL mixed standard was prepared from the individual stock solutions with a final solvent composition of 10:90 (v/v), water/methanol.

Calibration curve preparation: Stock solutions were prepared in methanol and spiked into calibration solution 1 or 2, depending on compound-specific solubility. Calibration solutions were composed of 50:50 (v/v), water/ethanol with 3% acetic acid by volume (calibration curve 1) and 50:50 (v/v), water/ethanol (calibration curve 2). Thirteen of the 16 Irganox compounds were prepared as described for calibration curve 1. The final calibration curves ranged from 0.025 to 50 ng/mL.

Food simulant spikes: Compounds were spiked into each of the 4 food simulants to yield final in-sample concentrations of 0.10, 1.00 and 2.5 ng/mL but were then diluted using the appropriate solvent in a 1:2 ratio to ensure complete solubility (Table 1). Therefore, the final in-vial concentrations were 0.033, 0.33 and 0.83 ng/mL. The food simulant spike samples were quantified using both calibration curves 1 and 2.

Food packaging material extract preparation: A 1 g sample of a plastic food packaging material (yogurt container) was weighed into each 10 mL glass centrifuge tube and 10 mL of each simulant was added (Table 1). The tubes were vortexed for 5 minutes and incubated at 40°C for 24 hours. Samples were filtered through a 0.22 µm syringe filter and diluted using the appropriate dilution solvent in a 1:2 ratio (Table 1).

Post-extraction spike of food packaging material: Extracts were prepared as described above. The final extract was spiked with the mixed Irganox standard to yield an in-sample concentration of 10 ng/mL. The resulting solution was then diluted with the appropriate solvent in a 1:2 ratio (Table 1).

Table 1. Food simulants and dilution solvents used to evaluate plastic materials or articles intended for food contact based on the EU commission regulation (10/2011).

Food simulant	Dilution solvent
3% Acetic acid (w/v)	*100% Ethanol*
10% Ethanol (w/v)	*50 % Ethanol with 3% acetic acid*
20% Ethanol (w/v)	*50 % Ethanol with 3% acetic acid*
50% Ethanol (w/v)	*Samples were injected without dilution*

Chromatography: The ExionLC AD system was used with a Phenomenex Luna Omega Polar C18 analytical column (C18 100Å, 3 µm, 100 x 4.6 mm). In addition, an Agela Ghost Hunter column (50 x 4.6 mm) was used as a delay column to separate the analytical peak from the LC pump contamination. The LC gradient conditions used are shown in Table 2. Mobile phase A was water with 10mM ammonium formate and 0.1% formic acid by volume. Mobile phase B was methanol. The flow rate was 0.8 mL/min and the column oven temperature was 40°C.

Table 2. LC gradient used for the separation of 16 Irganox compounds.

Time (min)	%A	%B
0.01	*10*	*90*
1.0	*10*	*90*
2.0	*2*	*98*
10.5	*2*	*98*
10.6	*10*	*90*
12.0	*10*	*90*

Mass spectrometry: Samples were analyzed using the SCIEX QTRAP 6500+ system with electrospray ionization (ESI) and polarity switching. Data were acquired using the Scheduled MRM algorithm. The target scan time was 0.80 sec for positive polarity and 0.30 sec for negative polarity. The optimized source and gas parameters and compound-specific MRM parameters used are shown in Tables 3 and 4, respectively.

Table 3. Optimized source and gas parameters.

Parameter	Value
Curtain Gas	*40 psi*
CAD Gas	*Medium*
Ion Spray Voltage	*4500/-4500 V*
Temperature	*500°C*
GS1	*90 psi*
GS2	*35 psi*

Data processing: All data were processed using SCIEX OS software, version 2.1.6. For the consistency with the EU regulations, concentrations are reported on an in-sample basis which is representative the method sensitivity, whereas the in-vial concentrations represent the instrument sensitivity.

Table 4. Optimized compound-specific parameters used for analysis.

Compound	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Irganox PS 800	*515.4*	*329.2*	*150*	*20*	*13*
Irganox 1010	*1194.8*	*785.4*	*130*	*73*	*13*
Irganox 1330	*792.6*	*219.1*	*125*	*37*	*12*
Irganox MD1024	*553.4*	*237.1*	*120*	*38*	*10*
Irganox 565	*589.3*	*250.0*	*160*	*62*	*15*
Irganox 5057	*394.3*	*134.1*	*195*	*50*	*10*
Irganox 3125	*1059.6*	*762.4*	*100*	*44*	*14*
Irganox 1098	*637.5*	*581.4*	*130*	*32*	*11*
Irganox 259	*656.6*	*415.3*	*80*	*34*	*10*
Irganox 1081	*359.0*	*139.1*	*40*	*28*	*8*
Irganox E201	*431.1*	*165.1*	*120*	*37*	*10*
Antioxidant 425	*386.3*	*135.1*	*20*	*26*	*16*
Irganox 3114	*801.5*	*784.5*	*88*	*20*	*12*
Irganox 1425	*327.1*	*299.1*	*-115*	*-33*	*-11*
Irganox 1035	*641.3*	*423.2*	*-130*	*-42*	*-13*
Irganox 1520	*423.2*	*145*	*-60*	*-21*	*-11*

Chromatographic separation

Good separation was achieved for the 16 Irganox compounds using an optimized chromatography method (Figure 1). A fast polarity switching method was used to acquire data for both positive and negative ion compounds in a single injection. A delay column was introduced after the mixing chamber to reduce the background interference from the mobile phases, thereby improving the method detection limits.

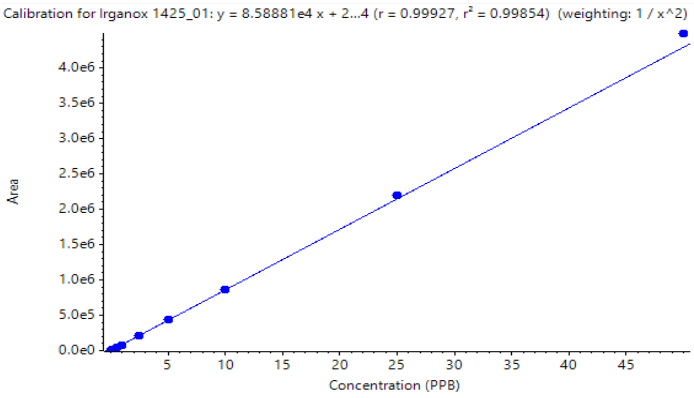

Figure 2. Illustrative calibration curve for Irganox 1425. The calibration curve was prepared using 50:50 (v/v), water/ethanol with 3% acetic acid (calibration curve 1) as diluent over the range of 0.025-50 ng/mL. An r^2 value >0.99 was achieved.

Calibration curve sensitivity and linear dynamic range

Calibration curves were generated for all compounds analyzed across a concentration range of 0.025 to 50 ng/mL. Standards were prepared in the calibration 1 diluent (50:50 (v/v), water/ethanol with 3% acetic acid by volume), unless specified. Table 5 shows the accurate quantification achieved across this range with an r^2 value >0.99. The lower limit of quantification (LLOQ) is represented by the lowest standard analyzed in the calibration range and this value varied from 0.025 to 0.5 ng/mL across Irganox compounds. Figure 2 shows an example calibration curve using the quantifier transition for Irganox 1425, which demonstrates the linear dynamic range of 0.025 to 50 ng/mL and r^2>0.99.

Table 5. Linearity range and correlation coefficient (r^2) achieved for the 16 Irganox compounds.

Compound	Linearity range (ng/mL)	Correlation coefficient (r^2)
Irganox PS 800	*0.250–50*	*0.991*
*Irganox 1010**	*0.500–50*	*0.993*
Irganox 1330	*0.050–10*	*0.990*
Irganox MD1024	*0.100–50*	*0.995*
*Irganox 565**	*0.025–50*	*0.992*
Irganox 5057	*0.050–25*	*0.993*
Irganox 3125	*0.100–25*	*0.990*
Irganox 1098	*0.100–50*	*0.999*
Irganox 259	*0.025–50*	*0.996*
Irganox 1081	*0.500–50*	*0.998*
Irganox E201	*0.250–50*	*0.996*
Antioxidant 425	*0.050–50*	*0.997*
*Irganox 3114**	*0.500–50*	*0.992*
Irganox 1425	*0.025–50*	*0.999*
Irganox 1035	*0.025–50*	*0.991*
Irganox 1520	*0.050–25*	*0.991*

Note: *All Irganox compounds were quantified using calibration curve 1 (50:50 (v/v), water/ethanol with 3% (v/v) acetic acid) except for those denoted with an asterisk, which were quantified using calibration curve 2 (50:50 (v/v), water/ethanol).*

Accurate and precise quantification of Irganox compounds in food simulant spikes using a single solvent calibration curve

Excellent accuracy (70-130%) and precision (%CV <15%) were achieved for 13 of the 16 Irganox compounds in all 4 food simulant spikes when quantified with the calibration curve 1 solutions (Table 6). The remaining 3 compounds (Irganox 1010, 565 and 3114) showed good accuracy and precision with either calibration curve 1 or calibration curve 2 solutions, depending on the food simulant matrix. Excellent precision based on 6 replicates was achieved at the LLOQ of all compounds spiked in each food simulant. These results demonstrate that good data quality can be achieved for most Irganox compounds using a single solvent curve across the diversity of food simulant matrices specified in the EU regulations. Removing the need to run individual matrix-matched curves can save significant sample preparation and analysis time.

Food packaging material spikes

Overall, the 10 ng/mL post-extraction spikes of the representative plastic food material (for example, yogurt container) showed good accuracy among the 4 food simulants, indicating minimal quantitative bias due to the matrix (Figure 3). The individual data points in Figure 3 represent the average of 6 replicates per Irganox compound. The 10% and 20% ethanol food simulants showed 70-130% accuracy for all compounds except for Irganox 1010 in the 10% ethanol simulant. The 50% ethanol and 3% acetic acid extracts showed 3 and 4 Irganox compounds outside the 70-130% acceptable range, respectively. No Irganox compounds were detected in the yogurt container, prior to spiking.

It is recommended to analyze the Irganox compounds with accuracies that failed to meet the 70-130% accuracy criteria using matrix-matched calibration or with mass-labeled internal standards. Overall, these findings demonstrate that the majority of the Irganox compounds in the 4 food simulants can be accurately quantified without the need for internal standards.

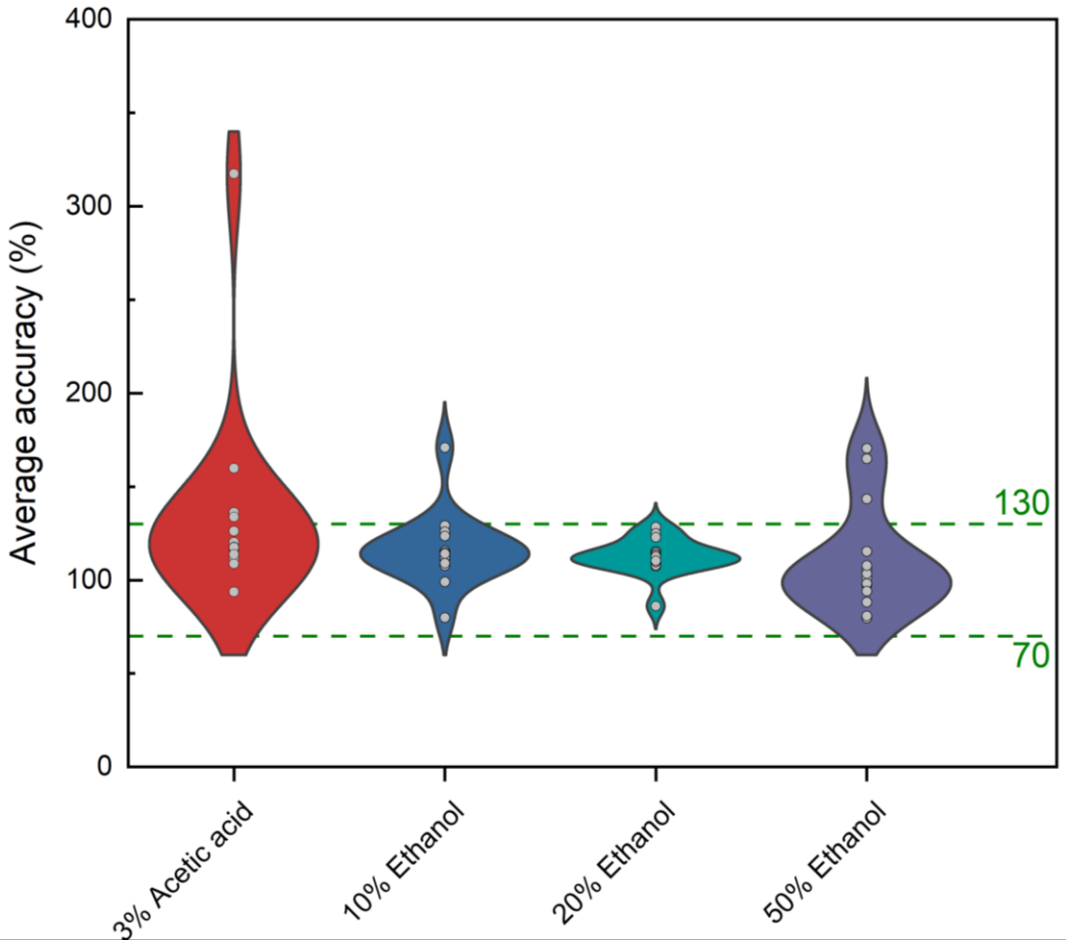

Figure 3. Average accuracy (n=6) of post-extraction spikes from the plastic yogurt container. Final extracts were spiked to yield 10 ng/mL in-sample and then diluted 2:1 with the compatible diluent solvent. Dots represent averages for individual Irganox compounds and the curve width represents approximate frequency of data points.

Table 6. Average accuracy and %CV (n=6) for all 16 Irganox compounds at the lowest measured concentration. Concentrations are reported on an in-sample basis.

Simulant	3% Acetic acid		10 % Ethanol		20 % Ethanol		50% Ethanol	
Analyte	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)
Irganox PS 800	1.00	119 (11)	1.00	120 (13)	1.00	124 (13)	1.00	94 (5.6)
Irganox 1330	1.00	114 (3.0)	1.00	121 (2.8)	1.00	120 (3.1)	0.025	112 (3.3)
Irganox MD1024	0.10	113 (1.9)	0.10	108 (6.8)	0.10	109 (2.7)	0.025	100 (5.9)
Irganox 5057	0.10	115 (3.7)	0.10	117 (8.4)	0.10	122 (4.0)	0.025	122 (9.1)
Irganox 1098	0.10	118 (12)	0.10	115 (7.9)	0.10	107 (1.5)	0.025	100 (1.8)
Irganox 259	0.10	114 (2.4)	0.10	111 (2.2)	0.10	110 (2.4)	0.025	108 (0.8)
Irganox 1081	1.00	105 (13)	1.00	101 (11)	1.00	112 (11)	1.00	105 (5.0)
Irganox E201	1.00	103 (4.8)	2.50	113 (3.1)	2.50	113 (9.7)	1.00	125 (3.4)
Antioxidant 425	0.10	95 (13)	0.10	88 (6.4)	0.10	86 (12)	0.025	70 (3.3)
Irganox 1425	1.00	108 (4.1)	1.00	112 (2.9)	1.00	108 (3.4)	0.10	79 (11)
Irganox 1035	0.10	111 (9.6)	0.10	109 (11)	1.00*	95 (5.3)	0.025	106 (13)
Irganox 3125	1.00	106 (6.2)	1.00	104 (5.6)	1.00	103 (5.6)	0.10	91 (6.9)
Irganox 1520	1.00	117 (3.0)	1.00	103 (2.5)	1.00	105 (2.3)	0.025	117 (6.0)
Irganox 1010	2.50*	106* (8.4)	2.50	106 (12)	2.50	96 (2.4)	1.00*	118* (7.3)
Irganox 565	1.00	96 (7.9)	1.00*	95* (2.6)	1.00*	85* (7.4)	0.025*	90* (5.9)
Irganox 3114	1.00*	114* (5.4)	1.00*	121* (2.6)	1.00*	117* (3.3)	1.00*	89* (2.9)

Note: *All Irganox compounds were quantified using calibration curve 1 (50:50 (v/v), water/ethanol with 3% (v/v) acetic acid) except for those denoted with an asterisk, which were quantified using calibration curve 2 (50:50 (v/v), water/ethanol).*

Conclusions

- A fast polarity switching method was developed to analyze 16 Irganox compounds in a single method
- The SCIEX QTRAP 6500+ system provided excellent sensitivity, reproducibility and linearity when quantifying Irganox compounds in different food simulants and post-extraction spikes in a plastic food container
- Two solvent-based external calibration curves were used to evaluate Irganox compounds in different simulants. Matrix-matched calibration curves were not required.
- R^2 values >0.99 were achieved for all compounds with %CV <15% across 6 replicates at the lowest measured concentration in all simulants, meeting the EU regulation requirements

References

1. Commission regulation (EU) No 10/2011 of 14th January 2011 on plastic materials and articles intended to come into contact with food (EUR-Lex - 32011R0010 - EN - EUR-Lex (europa.eu).

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