

Routine determination of phthalates in vegetable oil by single quadrupole GC-MS

Authors

Aaron Lamb, Dominic Roberts,
and Cristian Cojocariu
Thermo Fisher Scientific, Runcorn, UK

Keywords

Food safety, phthalates, trace analysis, gas chromatography, ISQ 7000, single quadrupole mass spectrometry, selected ion monitoring, vegetable oil, sensitivity, advanced electron ionization, AEI

Goal

To evaluate the suitability of the new Thermo Scientific™ ISQ™ 7000 GC-MS system, configured with the highly sensitive Advanced Electron Ionization (AEI) source, for the analysis of phthalates. Method selectivity, linearity, recoveries, and robustness were assessed using a challenging vegetable oil matrix.

Introduction

Phthalates (phthalate acid esters, PAEs) are a class of chemicals that are used mainly as plasticizers in various industries. Plasticizers are not chemically bound to their native polymer and therefore can leach into food from packaging materials in significant amounts.¹ Due to their lipophilic nature, phthalates are highly likely to be found in fat containing foods including cooking oils. The most important congener is di-(2-ethylhexyl)-phthalate (DEHP), which accounts for about 50% of the world production of phthalates (Figure 1).¹

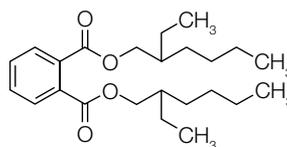


Figure 1. Chemical structure of the most prevalent phthalate, di-(2-ethylhexyl)-phthalate).

Previously, phthalates were believed to be non-toxic to humans, but now are classified as endocrine disruptors with associated adverse health effects and with links to autism in children.^{2,3} Recent cases of food contamination include the discovery that DEHP was intentionally added to sports drinks, fruit juice, tea beverages, and other food products as a clouding agent.⁴ Vegetable oils in the US and EU consumer markets have been found to contain phthalates.⁵ As a result, the European Food Safety Authority (EFSA) panel on food additives, processing aids, flavorings, and materials in contact with food has undertaken evaluations of the safety of food contact materials (FCM), as well as assessments on other substances used in food. In 2012 the EFSA set limits for phthalates in FCMs at 0.1%. Also, China and Taiwan have set limits in food products at 1 part per million (ppm), corresponding to 1000 µg/kg.

Sensitive and robust methods for the analysis of phthalates in food are clearly needed to protect the end consumer from food adulteration and phthalate migration from FCMs. One of the major challenges for laboratories that will be required to test for phthalates in food commodities is the analysis of fatty matrices such as cooking oils. These are complex mixtures of triacyl

glycerides that are difficult to chromatograph and present a challenge to the selectivity, sensitivity, and robustness for GC-MS analysis.

In this work, the analytical performance of a new single quadrupole GC-MS system using the Advanced Electron Ionization (AEI) source was tested.

Experimental

Calibration standard preparation

Vegetable oil was purchased from a local store. To test the limit of detection (LOD) / limit of quantification (LOQ) and assess the linearity, individual phthalate solvent standard solutions (LGC Ltd, UK) were prepared by spiking GC-grade n-hexane with calibration solutions prepared at 100-fold increased concentration in n-hexane. Nine calibration levels for 13 phthalate compounds were prepared: 0.5, 1.0, 2.5, 5.0, 10, 25, 50, 100, and 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil).

Sample preparation

Samples of vegetable oil were spiked prior to extraction at three concentration levels: 5, 25, and 50 µg/kg (Figure 2). GC and MS system parameters are listed in Tables 1 and 2. Consumables are listed in the Appendix.

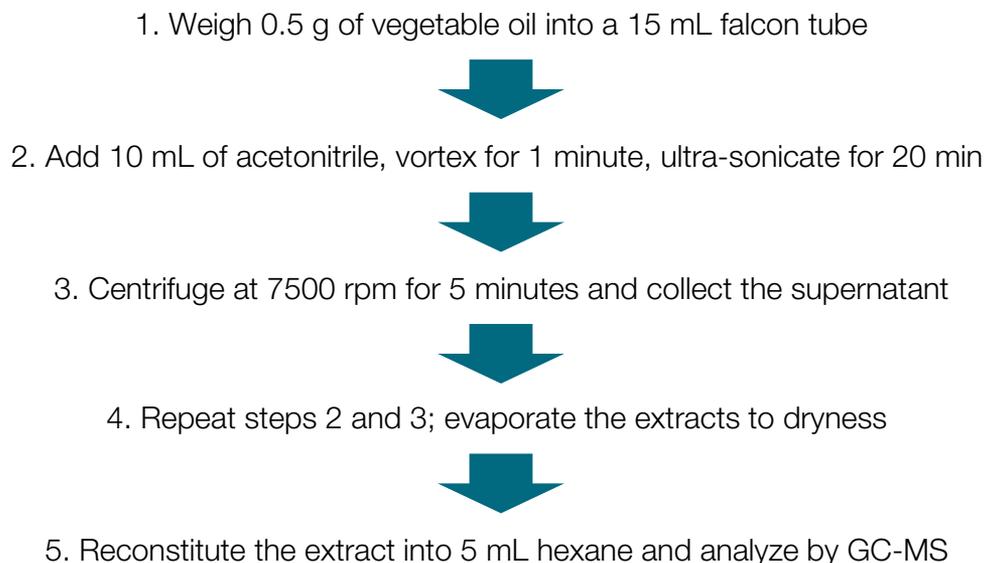


Figure 2. Vegetable oil sample preparation.

Table 1. Thermo Scientific™ AS 1310 autosampler and Thermo Scientific™ TRACE™ 1310 GC oven parameters.

Instrument conditions		
Autosampler parameters		
Fill strokes	10	
Air volume	1.0 µL	
Sample wash	2	
GC inlet parameters		
Injection volume	1 µL	
Injection mode	Splitless	
Temperature	300 °C	
Split flow	80.0 mL/min	
Splitless time	1.0 min	
Purge flow	5.0 mL/min	
Flow mode	Constant flow (1.0 mL/min)	
Carrier gas	Helium	
GC oven settings		
Ramp rate (°C)	Target value (°C)	Hold time (min)
0	100	1.0
20	190	0.0
10	280	5.0
30	320	10.0

See Appendix for consumables used.

Results and discussion

To assess the selectivity, sensitivity, linearity, and robustness of the ISQ 7000 GC-MS system configured with the AEI source, a complex vegetable oil matrix was selected. An example of the complexity of the total ion current (TIC) chromatography for full scan (FS) data of vegetable oil is shown in Figure 3.

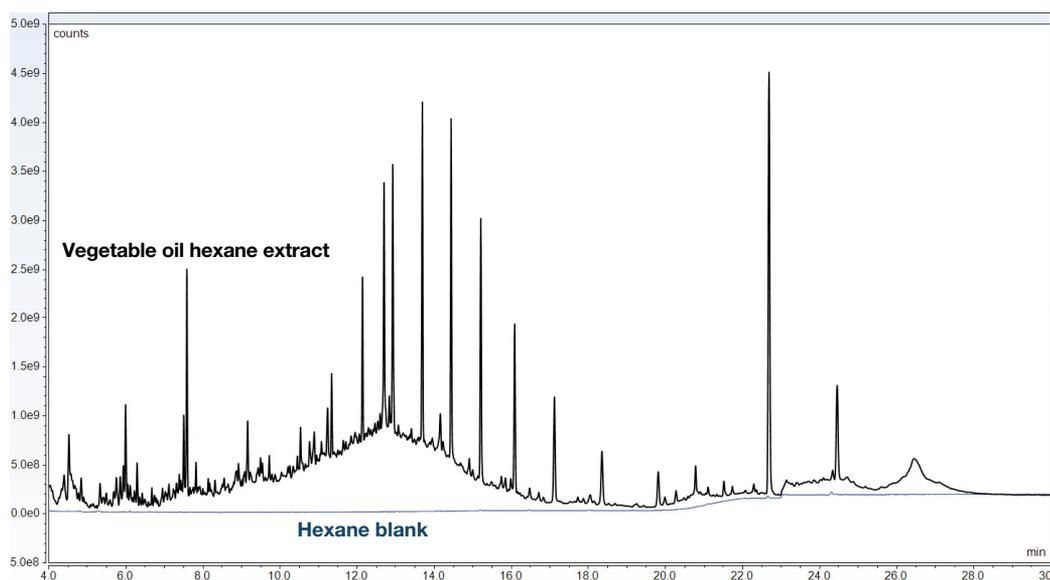


Figure 3. Example showing the cleanliness of the n-hexane blank and the complexity of the vegetable oil n-hexane extract overlays.

Table 2. ISQ 7000 GC-MS system parameters.

MS conditions				
Transfer line temperature	300 °C			
Ion source temperature	350 °C			
Acquisition mode	Timed (SIM)			
Ionization mode	EI (45 eV)			
Emission current	10 µA			
Minimum peak width	3 s			
Minimum scans/peak	12			
Name	RT (min)	(SIM) m/z		
		Quant	Qual 1	Qual 2
DMP	5.8	163	194	77
DEP	6.7	149	177	121
DAP	7.8	149	41	132
DIBP	8.8	149	205	223
DBP	9.6	149	223	205
DPP	11.1	149	237	219
DHXP	12.6	251	149	104
BBP	12.7	149	91	206
DCHP	14.0	149	167	249
DEHP	14.1	149	167	279
DNOP	15.6	149	279	167
DINP	16.1	293	149	167
DIDP	17.7	307	149	167

When using full scan acquisition, it is difficult to selectively detect phthalates such as DEHP from the background ions. In contrast, by using selective ion monitoring mode (SIM), a significant selectivity and sensitivity improvement is obtained (Figure 4).

Given the complexity of the chromatogram, analysis of phthalates in vegetable oil was carried out using timed-SIM. Timed-SIM mode is an excellent choice for quantitative GC-MS analysis because it allows the

detection of analytes with increased sensitivity. In SIM mode, data are gathered only for masses of interest rather than a full mass range, and the optimization of both scan rate and dwell time can be performed automatically using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software by inputting the desired number of points across the narrowest peak of interest and its peak width in seconds. This leads to greatly increased sensitivity and lower limits of quantification.

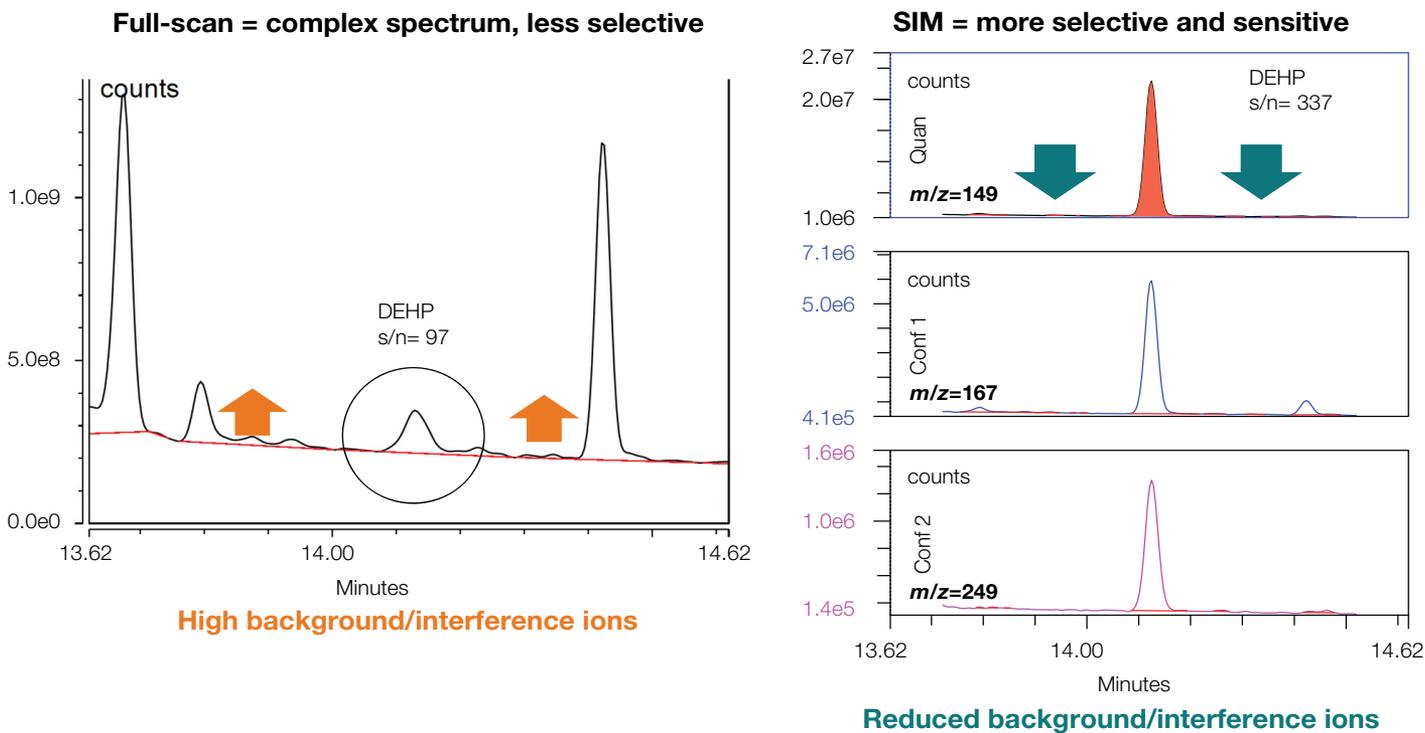


Figure 4. Example of selectivity and sensitivity obtained for DEHP when using SIM and FS for a vegetable oil hexane extract.

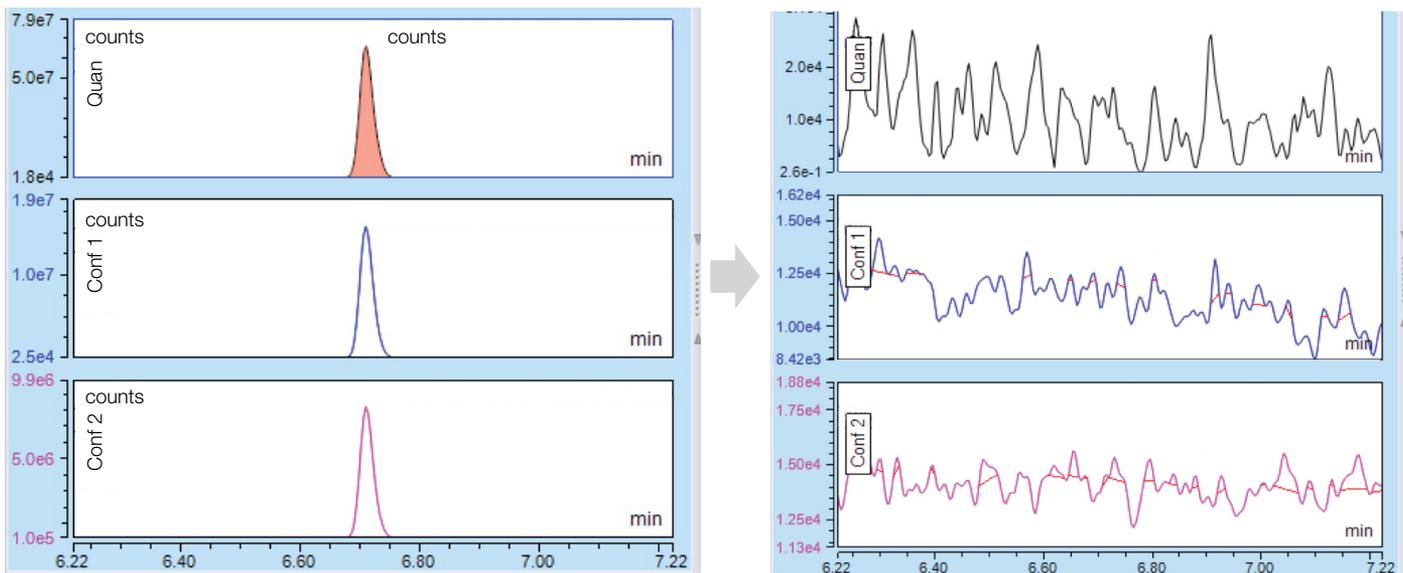


Figure 5. Example of DEP analyzed in a 50 ng/mL solvent standard and in a consecutive n-hexane blank injection showing no compound carry-over.

Overcoming contamination in phthalates analysis

As phthalates are ubiquitous compounds, during routine GC-MS analysis many sources of contamination can arise, such as potential plastic contact materials (polyethylene terephthalate). This problem is exacerbated by the high vapor pressures and chemical properties of phthalates increasing their persistence in the inlet, transfer line, and ion source if instrumental conditions are non-optimized. To avoid such contamination and to reduce potential carry-over from injection to injection, optimal consumable choice and method parameters are critical. This includes using PTFE/siloxane vial closures and bleed temperature optimized (BTO) inlet septa, as well as using optimized wash, inlet, and MS conditions (Figure 5).

Enhanced selectivity using SIM

Using SIM acquisition mode, selective and sensitive detection of phthalates in the food matrices was achieved. An example of SIM chromatograms including a stacked chromatogram (quantitation ion and 2x confirmation ions) at 0.5 ng/mL (5 µg/kg) level are shown in Figure 6 for the vegetable oil sample.

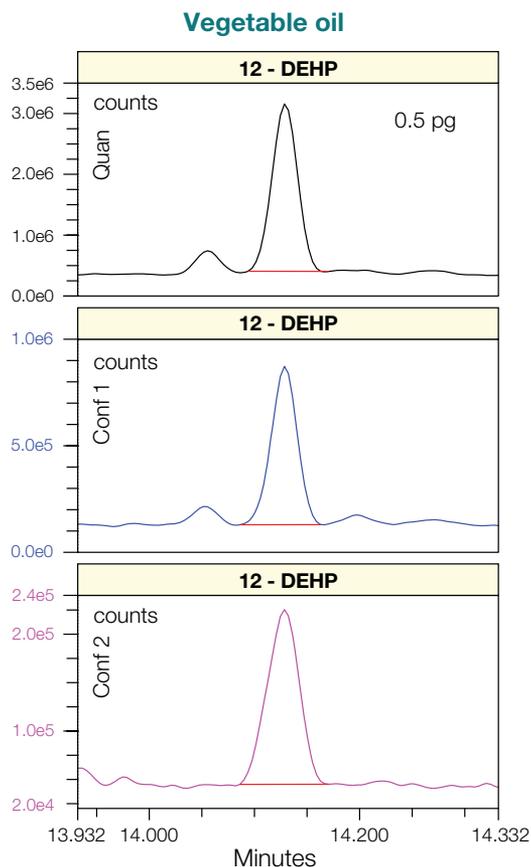


Figure 6. Example of SIM chromatograms for DEHP spiked at 0.5 ng/mL (5 µg/kg) in a vegetable oil n-hexane extract showing excellent sensitivity. On-column amount is also annotated.

Determination of LOD and LOQ

Phthalate residues in food products currently have no regulatory limits in the EU. However in this application levels of 5–25 µg/kg were achieved.

To practically assess the method's limit of detection, 18 replicate injections (of standards around the LOQ for each component) were performed. The instrumental detection limit (IDL) for each individual compound was then calculated by taking into account the injected amount, % RSD, and t-score of 2.567, corresponding to 17 degrees of freedom at 99% confidence (Table 3).

In addition to this, the LOQ was determined as the lowest concentration level of phthalates with a peak area repeatability of < 15% RSD and ion ratios within < ±15% of the expected values calculated as an average across a calibration curve ranging from 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). Based on these criteria, the estimated LOQs for compounds ranged from 5 to 25 µg/kg. An example of LOQ determination for the most difficult matrix is shown in Table 4.

Table 3. Estimated IDLs and absolute peak area repeatability (as % RSD) for phthalates determined from n=18 injections of a lowest concentrated standard where the peak area % RSD was lower than 15%.

Estimated IDL levels			
Component	Level injected (µg OC*)	% RSD	IDL (µg OC*)
DMP	0.1	4.1%	0.01
DEP	0.1	11%	0.03
DAP	0.1	7.8%	0.02
DIBP	0.1	2.7%	0.01
DBP	0.1	3.2%	0.01
DPP	0.1	5.7%	0.01
DXHP	1.0	9.2%	0.24
BBP	0.1	14%	0.04
DCHP	25	4.5%	3.0
DEHP	0.1	5.8%	0.01
DNOP	0.1	7.6%	0.02
DINP	25	2.4%	1.6
DIDP	25	3.0%	1.9

* OC = on column

Table 4. LOQ, absolute peak area, and ion ratio stability for targeted phthalates in vegetable oil (n=3 injections) at 5.0 µg/kg and at 25 µg/kg.

Estimated LOQ levels					
Compound name	LOQ (µg/kg)	Peak area	Ion ratio	Peak area	Ion ratio
		% RSD 5 µg/kg	% RSD 5 µg/kg	% RSD 25 µg/kg	% RSD 25 µg/kg
DMP	5.0	1.0	2.2	0.9	2.9
DEP	5.0	0.3	4.6	1.2	3.2
DAP	5.0	7.3	7.7	1.3	2.4
DIBP	5.0	0.9	6.8	1.0	2.5
DBP	5.0	3.1	4.1	1.2	0.9
DPP	5.0	11	7.7	1.5	11
DXHP	5.0	7.9	1.7	0.6	2.7
BBP	5.0	0.4	6.5	1.5	0.4
DCHP	25	NA	NA	2.3	2.8
DEHP	5.0	6.9	1.6	1.1	1.9
DNOP	5.0	5.5	4.2	1.9	2.2
DINP	25	NA	NA	2.5	5.4
DIDP	25	NA	NA	2.8	4.7

With the innovative design of the new AEI source, less frequent source cleaning is required as the improved source geometry leads to increased ionization efficiency and a narrower ion beam. This means the source filament can be operated at a reduced emission current, which in turn means less ionization of complex matrices in the source. Additionally, the highly focused ion beam significantly reduces the risk of source contamination. These features make the AEI source extremely robust, extending the time before maintenance is required. The enhanced sensitivity of the new source also means that

the sample matrix can be diluted more or the split ratio can be increased, further reducing the amount of potential contamination to the GC flow path.

Linearity

Linearity was determined using n-hexane solvent phthalate standards at concentrations of 0.5–250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil extracts). All compounds showed excellent linear response with coefficient of determination $R^2 > 0.998$, and average response factor values across this calibration range were all below 10% (Figure 7).

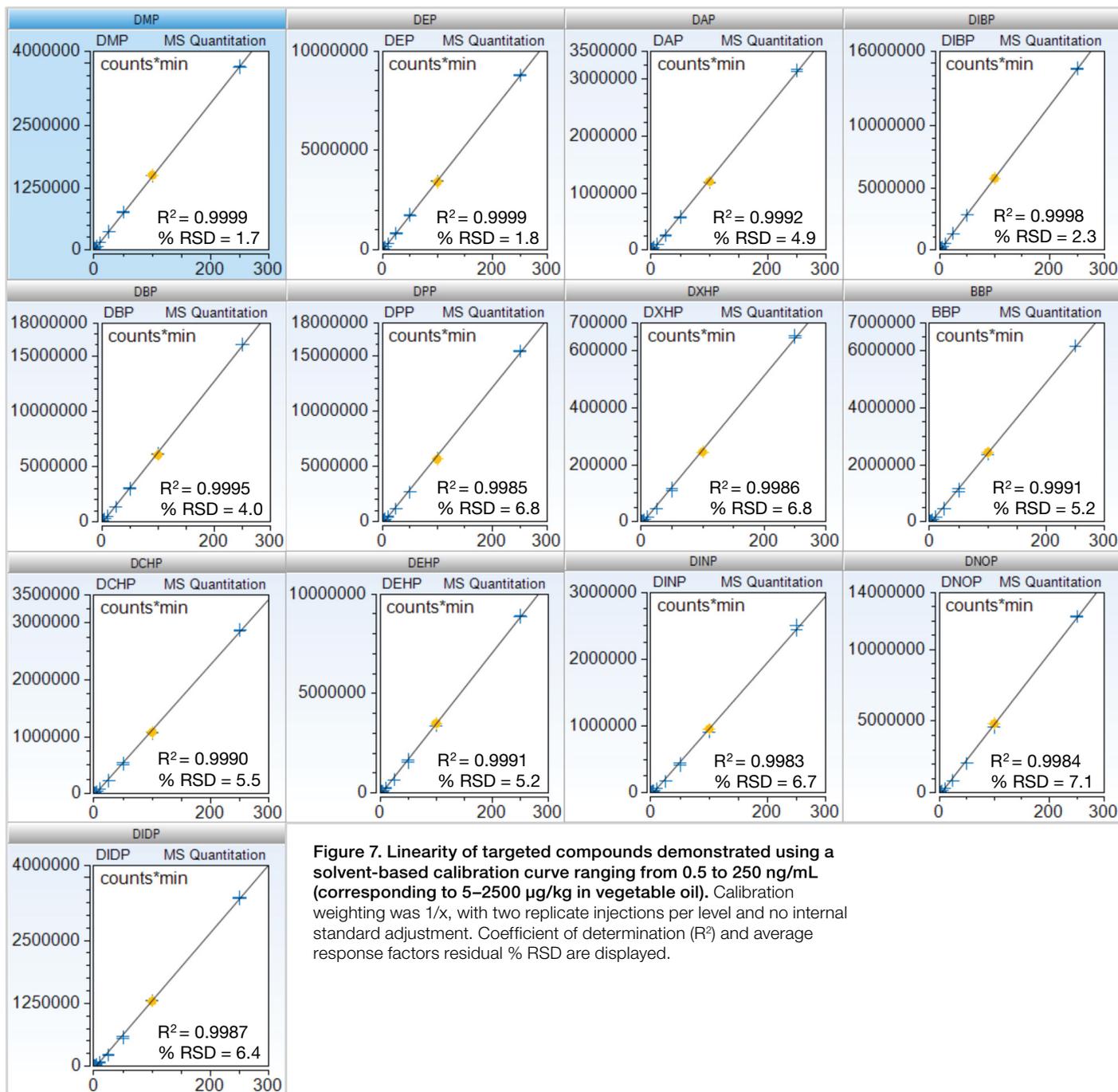


Figure 7. Linearity of targeted compounds demonstrated using a solvent-based calibration curve ranging from 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). Calibration weighting was 1/x, with two replicate injections per level and no internal standard adjustment. Coefficient of determination (R^2) and average response factors residual % RSD are displayed.

Method performance

The performance of the method was assessed by evaluating the recoveries in the pre- and post-spiked vegetable oil samples with a mixed phthalates standard at 5, 25, and 50 µg/kg. Three injections (technical replicates) per level were used and the results show average recovery values between 80% and 102% (Table 5).

Table 5. Recoveries (%) calculated for mixed phthalates pre-spiked in vegetable oil at three different concentration levels (5, 25, and 50 µg/kg) from n=3 injections. Recovery % RSD (n=3) is also shown.

Compound name	% Recovery 5.0 µg/kg spike level	% RSD (n=3)	% Recovery 25 µg/kg spike level	% RSD (n=3)	% Recovery 50 µg/kg spike level	% RSD (n=3)
DMP	101	1.7	98	1.9	104	5.2
DEP	102	1.8	98	4.8	100	3.7
DAP	97	1.7	95	0.8	99	3.0
DIBP	101	4.1	97	2.3	99	4.3
DBP	100	3.0	97	1.2	100	3.2
DPP	97	1.4	96	2.2	97	1.4
DXHP	97	5.9	91	0.5	95	0.6
BBP	92	3.4	93	0.3	91	2.0
DCHP*	NA	NA	91	1.3	84	4.8
DEHP	96	2.2	91	6.7	93	5.5
DNOP	93	3.9	93	2.6	97	0.5
DINP*	NA	NA	96	2.0	101	0.1
DIDP*	NA	NA	92	2.0	84	0.4

* The % recoveries were not calculated at the 5 µg/kg level as this was below the LOQ.

Conclusion

- The new innovative Thermo Scientific AEI source exhibits excellent sensitivity with unrivaled instrument detection limits of phthalate esters down to low ppt levels (0.01 ng/mL).
- Outstanding linearity for 13 phthalates analyzed was demonstrated over a range of 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). All compounds showed linear responses with coefficient of determinations $R^2 > 0.998$ average response factor RSDs < 10%.
- Compound recoveries demonstrated across three separate spiking levels were between 80% and 102%, well within the required method performance limits.

The ISQ 7000 GC-MS system configured with the AEI source provides unrivaled levels of sensitivity and robustness due to improved source geometry resulting in enhanced ionization efficiency and a narrower ion beam. This allows the user the flexibility to dilute their sample more, inject less, or use split methods while still being

able to achieve the required limits of detection. Reduced matrix load on the GC-MS system means reduced frequency of costly preventive instrument maintenance, such as consumable replacement and source cleaning, increasing the profitability and laboratory productivity.

References

1. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Official Journal of the European Union.
2. Kavlock, R.J.; Daston, G.P. DeRosa, C.; Fenner-Crisp, P.; Gray, L.E.; Kaattari, S., et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S.EPA-sponsored workshop. *Environ. Health Perspect.* **1996**, Aug;104 Suppl 4, 715–40. doi: 10.1289/ehp.96104s4715.
3. Clausen, P.A.; Hansen, V.; Gunnarsen, L.; Afshari, A.; Wolkoff, P. Emission of di-2-ethylhexyl phthalate from PVC flooring into air and uptake in dust: emission and sorption experiments in FLEC and CLIMPAQ. *Environ. Sci. Technol.* **2004**, *38*(9), 2531–7. doi: 10.1021/es0347944.
4. Yanga, J.; Hausera, R.; Goldman, R.H.; Taiwan food scandal: The illegal use of phthalates as a clouding agent and their contribution to maternal exposure, *Food and Chemical Toxicology.* **2013**; *58*, 362–368, doi.org/10.1016/j.fct.2013.05.010.
5. Serrano, S.E.; Braun, J.; Trasande, L.; Dills, R.; Sathyanarayana, S. Phthalates and diet: a review of the food monitoring and epidemiology data. *Environ. Health.* **2014**, *13*, 43.

Appendix A. Consumables list.

Consumable	Part Number
Column: Thermo Scientific™ TraceGOLD™ TG-5MS, 30 m × 0.25 mm × 0.25 μm	26098-1420
Injection septum: Thermo Scientific™ BTO, 11 mm	31303215-BP
Injection liner: Thermo Scientific™ LinerGOLD™, Single taper liner with quartz wool	453A2922-UI
Column inlet ferrules: Thermo Scientific™ 15% Graphite/85% Vespel 0.1–0.25 mm ID	290VA191
Column MS ferrules: Thermo Scientific™ 15% Graphite/85% Vespel 0.1–0.25 mm ID	290VT221
Spring loaded transfer line nut: Thermo Scientific™	1R120434-0010
Inlet base seal: Thermo Scientific™ 0.8 mm ID single column gold seal	290GA081
Injection syringe: Thermo Scientific™ 10 μL fixed needle syringe	365D0291
Solvent: Fisher Chemical™ Optima™ LC/MS Grade acetonitrile	Fisher Scientific A955-1
Solvent: Alfa Aesar™ Environmental Grade GC, >95%, n-hexane	Fisher Scientific AA42100K7
Conical centrifuge tubes: Corning™ Falcon™, 15 mL	Fisher Scientific 10136120
Conical centrifuge tubes: Corning™ Falcon™, 50 mL	Fisher Scientific 10788561
Vial: Thermo Scientific™ Virtuoso™, clear 2 mL kit with septa and cap	60180-VT402
Vial Identification System: Thermo Scientific™ Virtuoso™	60180-VT100

Find out more at thermofisher.com/ISQ7000

©2018 Thermo Fisher Scientific Inc. All rights reserved. Corning and Falcon are trademarks of Corning Life Sciences. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details.

AN10589-EN 0318S

ThermoFisher
SCIENTIFIC