Analysis of UV ink photoinitiators in packaged food by fast liquid chromatography at sub-ambient temperature coupled to tandem mass spectrometry

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Abstract

A fast method of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was developed for the analysis of eleven UV ink photoinitiators in packaged food. Chromatographic separation was achieved in a pentafluorophenylpropyl (PFPP) column at 5ºC and acetonitrile:25 mM formic acid-ammonium formate (pH 2.7) in gradient elution. To reduce sample treatment, a QuEChERS (quick, easy, cheap, effective, rugged and safe) method for the extraction and clean-up of UV photoinitiators in packaged foods was evaluated. Triple quadrupole working in H-SRM on Q1 mode was used for both quantitation and confirmation purposes and the most intense and selective transitions were chosen. Quality parameters of the developed QuEChERS LC-MS/MS method were established and applied for the analysis of photoinitiators in food packaged at ng kg^{-1} levels.

Keywords: Pentafluorophenyl propyl (PFPP) column, sub-ambient temperature, Tandem mass spectrometry, UV ink photoinitiators, QuEChERS, packaged food.

Introduction

The alert for food contamination by UV ink photoinitiators arose in Europe in November 2005, when the Italian Food Control Authority detected that the photoinitiator 2-isopropylthioxanthone (2-ITX) migrated into baby milk at concentrations ranging from 120 to 300 μg L^{-1}, resulting in the withdrawal from the market of more than 30 million liters of milk [1]. Since then, residues of other photoinitiators such as 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) or benzophenone (BP) have also been found in packaged food [2,3]. Photoinitiators are used as starters in the polymerization process to cure the ink by UV radiation. UV inks are used to print packaging materials such as multilayer laminates, rigid plastic, cardboard and paper. Although intermediate aluminum layers are commonly used to prevent the migration of ink components into food products, the unintentional transfer of printing ink components from the outer printed surface onto the food contact surface can occur when the printed material is rolled on spools or stacked during storage. Nowadays, these compounds are not regulated by specific EU legislation and maximum residue levels (MRL) in food are not established, but according to the European Food Safety Authority (EFSA) [4] the presence of some of them could be considered undesirable. Up to now, a maximum permitted amount for migration from packaging materials to packaged food has only been established for BP. This Specific Migration Limit (SML) was set at 600 μg L^{-1} for this photoinitiator [5].

In addition, the EU approved a Commission Regulation 2023/2006 [6], which sets out the rules for good manufacturing practice (GMP) for groups of materials and articles that are intended to come into contact with food. These materials should not transfer their constituents to food in quantities that might endanger human health or

bring about unacceptable changes in the composition of foodstuffs. Information about UV ink photoinitiators is also included in this document.

So far, in the literature there are few methods for the simultaneous analysis of UV ink photoinitiators. For analytical procedures, gas chromatography coupled to mass spectrometry (GC-MS) is the technique most frequently used to analyze this family of compounds. For instance, 2-ITX has been determined in milk samples [3,12,13], although other UV ink photoinitiators such as EHDAB, BP, 4,4'-bis(diethylamino)-benzophenone (DEAB) and 1-hydroxycyclohexyl phenyl ketone (HCPK) have been found in beverages [3,7,8]. Liquid chromatography (LC) with UV detection has been used to study the migration of some photoinitiators from printed food-packaging materials into food simulants or powdered milk [9,10]. In addition, some methods for the analysis of ITX in food and food packaging materials by LC with fluorescence detection have also been reported [11,12]. However, liquid chromatography-tandem mass spectrometry (LC-MS/MS) [2,3,13-18] has become popular for the analysis of UV ink photoinitiators, in order to confirm the identity of the analytes in food samples, following directive 2002/657/EC [19]. In general, most of these LC-MS/MS methods are devoted to the determination of ITX in food samples by reversed-phase liquid chromatography. The chromatographic separation of the two isomers (2-ITX and 4-ITX) can only be achieved by more selective columns such as a zirconium column and a pentafluorophenyl propyl (PFPP) column [15,17]. For the other UV ink photoinitiators, a few LC-MS/MS methods have been described using C18 columns [3,18], but with relatively long analysis times (above 20 min).

Due to the complexity of food matrices and the low concentration levels expected for UV ink photoinitiators in these samples, efficient preconcentration and

clean-up procedures are usually needed. Liquid-liquid extraction (LLE) [2,3,9,12,20] using acetonitrile or hexane is commonly used for the analysis of photoinitiators in liquid and fatty food samples. To reduce solvent consumption and improve selectivity, solid phase extraction (SPE) [14,17,18] is used as an alternative to LLE. Other extraction techniques such as pressurized liquid extraction (PLE) [2,11,13] and solid phase microextraction (SPME) [21] have also been used for the analysis of these compounds. Nowadays, the QuEChERS method (***Qu****ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe*) is a frequent and attractive alternative method for sample preparation in food analysis. The QuEChERS method is particularly popular for determination of polar, middle polar and non-polar pesticide residues in various food matrices [22-27], because of its simplicity, low cost, suitability for high throughput and relatively high efficiency with a minimal number of steps.

The aim of this work is to develop a fast liquid chromatography-tandem mass spectrometry method using a QuEChERS extraction method for the simultaneous determination of the most commonly employed UV ink photoinitiators in various ~~packaging~~ packaged foods.

2. Experimental

2.1. Materials and chemicals

The UV ink photoinitators (Figure 1), all of them of analytical grade, ethyl 4-dimethylaminobenzoate (EDMAB, 99%, CAS No. 10287-53-3), benzophenone (BP, 99%, CAS No. 119-61-9), 4,4'-bis(diethylamino)-benzophenone (DEAB, 99%, CAS No. 90-93-7), 4-benzoylbiphenyl (PBZ, 99%, CAS No. 2128-93-0), 2,4-diethyl-9*H*-

thioxanthen-9-one (DETX, 98%, CAS No. 82799-44-8), 1-hydroxycyclohexyl phenyl ketone (HCPK, 99%, CAS No. 947-19-3), 2-hydroxy-2-methylpropiophenone (HMPP, 97%, CAS No. 7473-98-5), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%, CAS No. 24650-42-8), 2-ethylhexyl 4-(dimethylamino)benzoate (EHDAB, 98%, CAS No. 21245-02-3), 2-isopropylthioxanthone (2-ITX, 99.7%, CAS No. 5495-84-1), 4-isopropylthioxanthone (4-ITX, 99.5%, CAS No. 83846-86-0) and 2-isopropyl-D7-thioxanthen-9-one (2-ITX-D_7 used as internal standard (I.S.), 99.5%, CAS No. 400-880-8822) were purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid (98-100%) was provided by Merck (Darmstadt, Germany). Anhydrous magnesium sulfate was obtained from Sigma (Steinheim, Germany), sodium chloride from Fluka (Steinheim, Sweden), and propylamino (PSA) bonded silica SPE bulk from Supelco (Gland, Switzerland). OASIS HLB cartridges (60 mg) purchased from Waters (Mildford, MA, US) were used for solid phase extraction. Supelco Visiprep and Supelco Visidry SPE vacuum manifold (Supelco) were used for SPE and solvent evaporation. LC-MS grade methanol (MeOH), acetonitrile (ACN) and water were purchased from Riedel-de Haën (Seelze, Germany).

Stock standard solutions of UV ink photoinitiators (1,000 mg kg^{-1}) were individually prepared by weight in methanol and stored at 4ºC. Working solutions were prepared weekly by appropriate dilution in acetonitrile:water (1:1) of the stock standard solution. Mobile phases were filtered using 0.22 μm nylon membrane filters (Whatman, Clifton, NJ, US) and sample extracts were filtered through 0.22 μm pore size Ultrafree-MC centrifuge filters (Millipore, Bedford, US).

Nitrogen (99.98% pure) supplied by Claind Nitrogen Generator N_2 FLO (Lenno, Italy) was used for the API source; and high-purity Argon (Ar1), purchased from Air

Liquide (Madrid, Spain), was used as a collision-induced gas (CID gas) in the triple quadrupole instrument.

2.2. Instrumentation

A liquid chromatography system (Accela; Thermo Fisher Scientific, San José, CA, US), equipped with a low-pressure quaternary pump, autosampler and column oven, was used. The chromatographic separation was performed in a pentafluorophenyl propyl column, Discovery® HS F5 (150 mm x 2.1 mm i.d., 3 µm particle size), from Supelco (Bellefonte, PA, US), using a gradient elution of acetonitrile (solvent A) and 25 mM formic acid-ammonium formate buffer at pH 2.7 (solvent B): 50% solvent A for 0.5 min followed by a linear gradient up to 80% solvent A in 2.5 min and an isocratic step for 3 minutes at this latter percentage. The flow-rate was 450 µL min^{-1} and the column temperature was held at 5^{o}C, providing a back-pressure ≤ 350 bar.

The liquid chromatography system was coupled with a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (Thermo Fisher Scientific), equipped with electrospray ionization (ESI) source and hyperbolic quadrupoles able to work in enhanced mass resolution mode (mass resolution at 0.1 *m/z* FWHM, full - with half maximum). Nitrogen (purity > 99.98%) was used as a sheath gas, ion sweep gas and auxiliary gas at flow-rates of 60, 20 and 40 a.u. (arbitrary units), respectively. The ion transfer tube temperature was set at 375^{o}C and electrospray voltage at +4 kV. Selected reaction monitoring (SRM) and highly-selective reaction monitoring (H-SRM) acquisition modes were used. In SRM mode, a mass resolution of 0.7 *m/z* FWHM on both Q1 and Q3 and a scan width of 0.01 *m/z* were used. In H-SRM mode, a mass resolution of 0.1 *m/z* FWHM on Q1 and a scan width of 0.01 *m/z* were employed, while

the other quadrupole operated at low resolution (0.7 *m/z* FWHM). Argon was used as collision gas at 1.5 mtorr and the optimum collision energy (CE) for each transition monitored (quantifier and qualifier) is shown in Table 1. The chromatogram was segmented into two windows, and two transitions for each compound with a dwell time of 50 ms and 1 µscan were monitored (Table 1). The Xcalibur software version 2.0 (Thermo Fisher Scientific, San Jose, CA, US) was used to control the LC/MS system and to process data.

To optimize both the ESI source and tandem mass spectrometry working conditions, 1 mg L^{-1} stock standard methanol solution of each compound was infused at a flow-rate of 3 µL min^{-1} using the syringe pump integrated in the TSQ instrument and mixed with the mobile phase (450 µL min^{-1}, acetonitrile:formic acid-ammonium formate buffer (70:30, *v/v*)), by means of a Valco zero dead volume tee piece (Supelco).

2.3. Sample treatment

2.3.1. Packaged foods

(i) For the QuEChERS method, sub-samples of 2.5 g were weighed into a 50 mL PTFE centrifuge tube (Serviquimia, Barcelona, Spain). 5 µL of 2-ITX-D_7 used as a surrogate (100 µg kg^{-1}) and 12 mL of acetonitrile were added. Then the mixture was shaken vigorously for 1 min using a vortex (Stuart, Stone, UK). After this step, 1.5 g of NaCl and 4 g of $MgSO_4$ were added to the extract and then shaken again for 1 min. The extract was then centrifuged at 2,500 rpm for 1 min using a Selecta Centronic centrifuge (Selecta, Barcelona, Spain) and 10 mL of the supernatant were transferred into a 15 mL graduated centrifuge tube that contained 250 mg of PSA (propylamine bonded silica SPE bulk) and 750 mg of $MgSO_4$. The mixture was energetically shaken for 1 min in a

vortex and centrifuged again at 3,700 rpm for 1 min. Finally, 8 mL of the supernatant were evaporated to dryness under a nitrogen stream and reconstituted in 500 µL acetonitrile:water (1:1, *v/v*). Prior to analysis, the extract was filtered through 0.22 µm-pore Ultrafree-MC centrifugal filters and transferred into an amber vial to prevent ~~analyte~~ analytes photodegradation. Finally, 10 µL of this extract were injected into the LC-MS/MS system.

(ii) An SPE method previously described in our research group for the analysis of ITX was also used [17]. Briefly, an aliquot of 2.5 g of homogenized sample was weighed into a 15 mL centrifuge tube; and 5 µL 2-ITX-D_7 (surrogated, 100 µg/kg) and 10 mL of acetonitrile were added. The resulting mixture was shaken for 30 min in a rotating shaker (Breda Scientific, Breda, Netherlands) and 1 mL of Carrez reagent 1 and 1 mL of Carrez reagent 2 were added. Then, the mixture was centrifuged at 3,500 rpm for 15 min with a Selecta Centronic centrifuge and 10 mL of the supernatant solution were diluted with 25 mL of LC-MS grade water and loaded into an OASIS® HLB (60 mg) SPE cartridge, which was previously conditioned with 6 mL of methanol and 6 mL of water. The analytes were eluted with 6 mL of acetonitrile. The collected fraction was evaporated to dryness under a nitrogen stream and was treated as described above for the QuEChERS method.

A total of 14 packaged food samples, including baby food, fruit juices, water, wine, two blank samples, a pineapple juice sample packaged in a plastic bottle and a baby food sample in a glass bottle obtained from local supermarkets (Barcelona, Spain), were analyzed. 2- and 4-ITX were quantified by isotope dilution using the deuterated standard (2-ITX-D_7), while the other photoinitiators were quantified by matrix matched calibration. In order to control possible contaminations method blank samples were analyzed.

2.3.2. Packaging materials in contact with food

Packaging materials in contact with food were processed by means of the method described by Sagratini *et al.* [3]. Briefly, the food carton was opened and the food content processed following the procedures described in Section 2.3.1., while the internal side of the packaging material was washed with LC-MS grade ultrapure water and then wiped. A 10 cm x 5 cm scrap of packaging polycoupled carton was cut into 1 cm^2 pieces, and then soaked in 50 mL of dichloromethane (amber glass bottle) for 24 h. After this, the organic solvent was collected and evaporated to 1 mL using nitrogen in a Turbovap® II Concentration Workstation (Zymark Corporation, Hopkinton, Massachusetts, USA), and finally evaporated to dryness using a Visidry vacuum manifold. The extract was reconstituted with 5 µL of 2-ITX-D_7 solution (100 µg kg^{-1}) and 495 µL of methanol:water 1:1 (*v/v*), filtered through 0.22 µm-pore Ultrafree-MC centrifugal filters and transferred into an amber injection vial. Finally, 10 µL of this extract were injected into the LC-MS/MS system.

3. Results and Discussion

3.1. Chromatographic separation

In this study, the fluorinated (pentafluorophenylpropyl) column (Discovery® HS F5) proposed in a previous paper for the chromatographic separation of the two ITX isomers (2-ITX and 4-ITX) [17] was used to separate eleven photoinitiators currently used in food packaging [1], using gradient elution based on a mobile phase of

acetonitrile/formic acid-ammonium formate buffer (25 mM, pH 2.7). First, the gradient elution was optimized and the best separation was obtained in 6 min using a linear gradient from 50% ACN to 80% in 2.5 min. However, under these conditions several co-elutions occurred: PBZ/DEAB, EDMAB/DMPA/BP and DETX/EHDAB. To improve the chromatographic separation, the effect of temperature was evaluated between 5ºC and 25ºC. As Figure 2 shows, chromatographic resolution improved significantly when temperature decreased and the best separation, especially for EDMAB/DMPA/BP, was at 5ºC (Figure 2C), providing resolutions better than 1.1 for these photoinitiators in less than 7 min, which led to the choice of this temperature for further studies. Temperatures below 5ºC were not evaluated because of the limitation on the minimum temperature allowed by the column oven controller (5ºC). To reduce the analysis time, flow-rate was increased up to 450 µL min^{-1} (Figure 2D). Under these working conditions, there was good chromatographic separation of all compounds in less than 5 min analysis time, generating a low backpressure (< 350 bar).

3.2. Liquid chromatography-mass spectrometry

The liquid chromatographic system was coupled to a triple quadrupole mass spectrometer using an ESI source in positive mode. For most of these compounds, the ESI (positive) full scan MS spectrum showed only the isotopic cluster corresponding to the protonated molecule $[M+H]^+$. However, for some of them (HMPP, HCPK, DMPA, DEAB), ions originated by in-source fragmentation were also observed (Table 1). The in-source fragmentation was especially important for DMPA, whose mass spectrum showed the in-source loss of a methoxy group as base peak, yielding the ion at *m/z* 225 $[M-CH_3O]^+$. The significant differences between structures of some of these

photoinitiators produced important differences in electrospray responses. Thioxanthone-based photoinitiators (2-ITX, 4-ITX and DETX) showed the highest response, followed by the alkyl-amino-based compounds (DEAB, EHDAB and EDMAB) (10 to 20 times lower) and the phenone-based compounds (BP, PBZ and DMPA) (20 to 200 times lower). HMPP and HCPK showed the lowest ionization efficiency.

The fragmentation of these compounds under tandem mass spectrometry conditions in the triple quadrupole was studied and the most intense and characteristic transitions were selected for both quantitative and confirmation purposes. For the correct product ion assignment, collision energy curves (5-80 V) were studied. The assignments for both precursor and monitored product ions for each compound are given in Table 1, which also gives the selected transitions and the optimal collision energies. Due to the differences in chemical structure of the compounds studied, it was difficult to select common transitions for the whole family. For ITX isomers (2- and 4-ITX) and DETX the most intense product ions corresponded to the loss of the alkyl chains. For ITX the ion originated from the consecutive losses of the alkyl chain and the CHO group (*m/z* 184) was also observed and selected as qualifier ion. The MS/MS spectrum of both BP and PBZ showed as a base peak the ion at *m/z* 105 corresponding to $[C_7H_5O]^+$ due to the α-cleavage of the carbonyl group. Another intense product ion corresponding to $[C_6H_5]^+$ was also observed and selected for confirmation. For compounds such as EHDAB and EDMAB, which contain both an amino and an ester group, the most intense product ions in the MS/MS spectra were generated by the consecutive losses of a methyl group and the alkyl chains of the ester group (*m/z* 151) and the methyl group together with the α-cleavage of the carbonyl group (*m/z* 134). The other photoinitiators, HCPK, HMPP and DMPA, showed a different fragmentation pattern because of the different functional groups in their structures. For HMPP, the

base peak in the MS/MS spectrum was the product ion at *m/z* 119, probably due to the consecutive neutral losses of water and olefin (C_2H_4), and the product ion at *m/z* 91, corresponding to the tropylium ion often found for aromatic compounds containing a benzyl unit, while HCPK showed the ion at *m/z* 105 originated by the α-cleavage of the carbonyl group, as occurred for BP and PBZ, and the neutral loss of water (*m/z* 187). Finally, for DMPA two abundant product ions were obtained from the fragmentation of the in-source fragment ion, the characteristic ion at *m/z* 105 as at *m/z* 197, due to the loss of a CO group.

To evaluate the performance of the fast LC-MS/MS method developed, instrument quality parameters such as limits of quantitation (ILOQ), linearity and run-to-run precision at two concentration levels, a low level close to the limit of quantitation (LOQ) and a medium level (HMPP: 3 mg L^{-1}; HCPK: 300 µg L^{-1}; other ink photoinitiators: 50-100 µg L^{-1}), were evaluated using selected reaction monitoring (SRM) acquisition mode. ILOQs (Table 2), based on a signal-to-noise ratio of 10:1, were calculated by the injection of 10 µL of UV ink photoinitiator standard solutions prepared at low concentration levels (background noise was determined manually around the compound retention time). Thioxanthone-based photoinitiators provided the lowest instrument ILOQs (0.06 to 0.09 µg L^{-1}), while compounds based on alkyl-amino groups (DEAB, EHDAB and EDMAB) and PBZ provided ten-times higher values (0.9 to 1.5 µg L^{-1}). Whereas phenones and HCPK showed ILOQ values between 15 and 30 µg L^{-1}, HMPP provided the highest ILOQ due to its lower ionization efficiency with ESI.

Calibration curves based on the peak area ration ($A_{compound}/A_{internal\ standard}$) (2-ITX-$D_7$ as I.S.) showed good linearity (correlation coefficient, r^2: >0.995). Moreover, linearity was also evaluated using statistical ANOVA analysis. For a 95% of confidence

level, *p*-values obtained (from 0.70 to 0.79) were higher than the confidence probability (0.05) so good linearity was observed in the working range. Run-to-run precision was also determined at two concentration levels (n=5) by LC-MS/MS (RSD < 6.6%).

3.3. Method performance

In this study we evaluated the applicability of a QuEChERS procedure for the analysis of UV ink photoinitiators in packaged foods. This method was compared with a SPE one previously applied for the analysis of ITX [17] in terms of sensitivity, ~~accuracy~~ trueness and precision. For these purposes two blank samples (pineapple juice and baby food) were spiked and submitted to both sample treatments. The results obtained for the baby food sample are summarized in Table 2.

In general, similar MLQs were obtained using both sample treatments for both matrices providing values down to μg kg^{-1} or even ng kg^{-1} for ITX and DETX (5 ng kg^{-1}), with the sole exception of HMPP, which showed the highest MLOQ value (666 μg kg^{-1}). To evaluate the run-to-run precision, six replicates of a blank sample spiked at the concentrations from 0.14 μg L^{-1} to 800 μg L^{-1}, except for HMPP (2.5 mg L^{-1}), (Table 2) were analyzed using both sample treatments. For day-to-day precision a total of 18 replicate determinations on 3 non-consecutive days (six replicates each day) were carried out. Similar relative standard deviations (%RSD) based on concentration were obtained for both SPE and QuEChERS, with values ranging from 1.9 to 5.1% (run-to-run) and from 6.5 to 10.1% (day-to-day). Good quantitation results, with a ~~accuracies~~ trueness (defined as % relative error) in the 81-98% range, were achieved. In addition, a statistical paired-sample comparison analysis was performed, based on the quantitation results obtained in both SPE and QuEChERS procedures. For a 95% confidence level,

the results were not significantly different (*p*-value of 0.33). Thus, the QuEChERS method provided similar results in terms of MLOQs, run-to-run and day-to-day precisions, and quantitation to results obtained for SPE, but with the additional advantage of being 12 times faster (per sample). These results mean that this method can be proposed for the fast analysis of UV ink photoinitiators in packaged food.

In addition, to improve sensitivity by minimizing interferences and background noise, enhanced mass resolution on precursor ions (H-SRM on Q1) was evaluated. For this purpose two blank samples (baby food and fruit juice) were spiked at a low concentration level (close to the quantitation limit) and analyzed by the QuEChERS method. Table 3 summarizes the peak intensity normalized to that of SRM mode and the signal-to-noise ratio obtained for each compound in pineapple and baby food, using SRM and H-SRM acquisition modes. It can be observed that the intensity of the compounds decreased when mass resolution increased, although a higher signal-to-noise ratio (S/N) was obtained due to a significant reduction in the background noise. This obtained MLOQs that were 1.25 to 30 times lower.

3.4. Application of the method

To evaluate the applicability of the QuEChERS LC-MS/MS method, 14 packaged foods (food commodities and baby foods) from Spanish supermarkets were analyzed. Their packaging materials were also analyzed in order to identify the UV ink photoinitiators used in the printing process, which might then be expected to be found in the packaged foods. Since BP can be used in the manufacture of plastic materials, analysis of blanks is relevant in order to detect contamination during the analytical procedure. In this study, no contamination was observed when analyzing method blank

samples. The results obtained showed that all the packaging materials contained between 4 and 8 photoinitiators, among which BP was always present at high concentrations (between 2 and 350 ng cm^{-2}). DMPA and the tertiary amine EHDAB were also found in many of the cartons analyzed, the first one at relatively high concentrations (0.2 – 1 ng cm^{-2}). Other photoinitiators such as EDMAB and DEAB were detected in some of the packaging materials, but at lower concentrations (0.005 – 0.6 ng cm^{-2}). The photoinitiator 2-ITX (0.005 – 0.1 ng cm^{-2}) was also detected in all the analyzed samples, while 4-ITX was only found in 3 of the 14 samples, but at concentration levels similar to 2-ITX levels. Finally, PBZ and DETX were found in only a few samples, probably due to less use, while HCPK and HMPP were not detected in any of the cartons analyzed. These results corroborate those reported in the literature [3,10] about the presence of these compounds in packaging materials where BP was found at relatively high concentrations in almost all samples analyzed.

The results obtained in the analysis of the 14 packaged foods are summarized in Table 4. These results showed that only 1-4 of the photoinitiators identified previously in the food packaging materials were detected in the foodstuff, with BP being the most abundant one, with concentrations ranging from 1.8 to 40 µg kg^{-1}. It must be pointed out that in two of the samples (baby food 3 and *gazpacho* 1) an important deviation (>42%) in the BP ion ratio was observed, which did not allow its confirmation in the samples (Directive 2002/657/EC) [19]. The presence of BP in all the samples could be due, not only to its use as a UV ink photoinitiator, but to its application in the production of polyethylene (PE) coating film [28], which is directly in contact with food. EDMAB and 2-ITX were also found in a relatively high number of samples (10 and 7 samples, respectively), but at lower concentrations (ng kg^{-1}) than BP. HMPP and HCPK were not detected in any sample, as expected from the results obtained in the analysis of the

carton materials, while the other photoinitiators such as DETX and EHDAB were detected in just a few samples at low ng kg^{-1} levels. For example, Figure 3 shows the LC-MS/MS chromatogram obtained for a pineapple juice sample and the corresponding packaging material. Among the seven photoinitiators detected in the corresponding carton material, only four of them, BP, DEAB and both ITX isomers, were detected in the pineapple juice sample.

In addition, it should be pointed out that the greater sensitivity provided by the H-SRM in Q1 acquisition mode detected and identified some of the analyzed compounds, which could not be detected when low-resolution SRM acquisition mode was used. For instance, 4-ITX in *gazpacho* 1, DETX in fruit juice 1 and EHDAB in baby food 3 and fruit juice 2 were quantified at low concentration levels by H-SRM.

Conclusions

In this study, a fast LC-MS/MS method was developed for the analysis of UV ink photoinitiators in packaged food. Good chromatographic separation, including ITX isomers, was achieved by using a pentafluorophenyl propyl (PFPP) column and operating at low temperature (5ºC). A flow rate of 450 µL min^{-1} was used to reduce the analysis time below 5.5 min without compromising the chromatographic efficiency. To reduce the sample treatment time, a QuEChERS method is proposed for the extraction and clean-up of UV photoinitiators in packaged foods.

The ESI mass spectra of this family of compounds were generally dominated by the $[M+H]^+$, except for DMPA, which showed important in-source fragmentation. For this compound, $[M-CH_3O]^+$ was selected as a precursor ion in MS/MS. H-SRM on Q1

is proposed as acquisition mode, since an up-to-30-fold improvement in MLOQs was obtained.

Several photoinitiators, BP, PBZ, DEAB, 2-ITX, 4-ITX, DETX, EHDAB, DMPA and EDMPA, were detected in the packaging materials, with benzophenone always present and at the highest concentration level. This photoinitiator was also detected in all packaged food samples, while the other compounds were only found in a few samples at low ng kg^{-1} levels. These results allow us to propose the QuEChERS LC-MS/MS as a simple, fast, robust and reproducible method for the analysis of photoinitiators in packaged food.

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Figure Captions

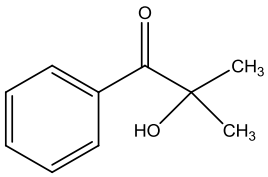
Figure 1. Chemical structures of photoinitiators.

Figure 2. Effect of column temperature on the separation of the eleven UV Ink photoinitiators. LC-MS/MS reconstructed chromatograms at (A) 25^{o}C, (B) 15^{o}C, (C) 5^{o}C at 300 μL min^{-1} and (D) 5^{o}C at 450 μL min^{-1}. Peak identification: 1, HMPP; 2, HCPK; 3, EDMAB; 4, DMPA; 5, BP; 6, PBZ; 7, DEAB; 8, 2-ITX; 9, 4-ITX; 10, EHDAB; 11, DETX.

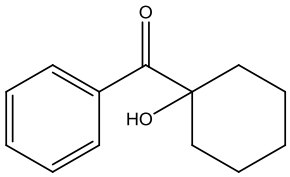
Figure 3. Analysis of (A) a packaging material containing a pineapple juice sample and (B) a pineapple juice sample. Conditions as indicated in the experimental section.

Figure 1

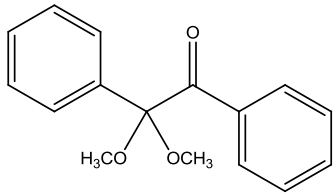
Figure 1



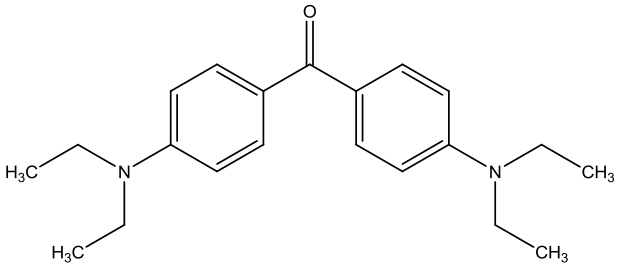
2-Hydroxy-2-methylpropiophenone
(HMPP)



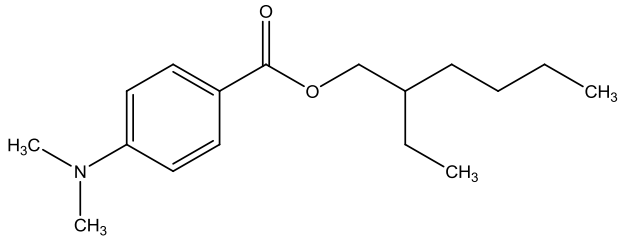
1-Hydroxycyclohexyl phenyl ketone
(HCPK)



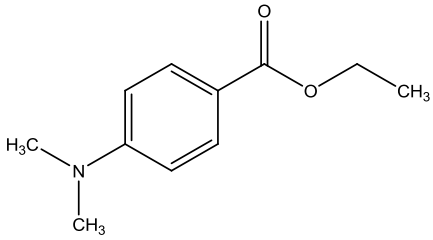
2,2-dimethoxy-2-phenylacetophenone
(DMPA)



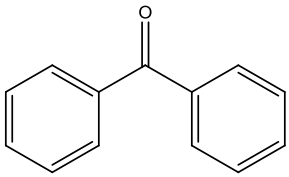
4,4'-Bis(diethylamino)-benzophenone
(DEAB)



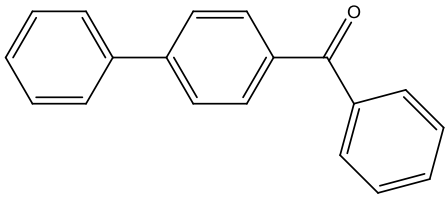
2-Ethylhexyl 4-(dimethylamino)benzoate
(EHDAB)



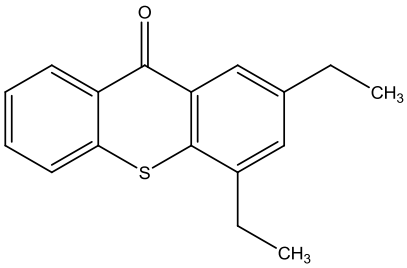
Ethyl 4-dimethylaminobenzoate
(EDMAB)



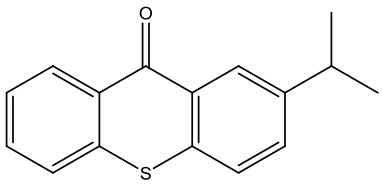
Benzophenone
(BP)



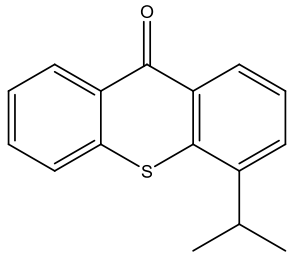
4-Benzoylbiphenyl
(PBZ)



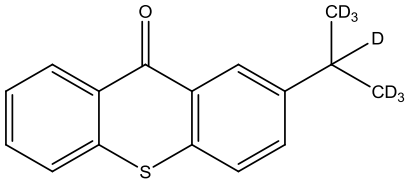
2,4-Diethyl-9*H*-thioxanthen-9-one
(DETX)



2-Isopropylthioxanthone
(2-ITX)



4-Isopropylthioxanthone
(4-ITX)



2-Isopropyl-D7-thioxanthen-9-one
(2-ITX-D7)

Figure 2

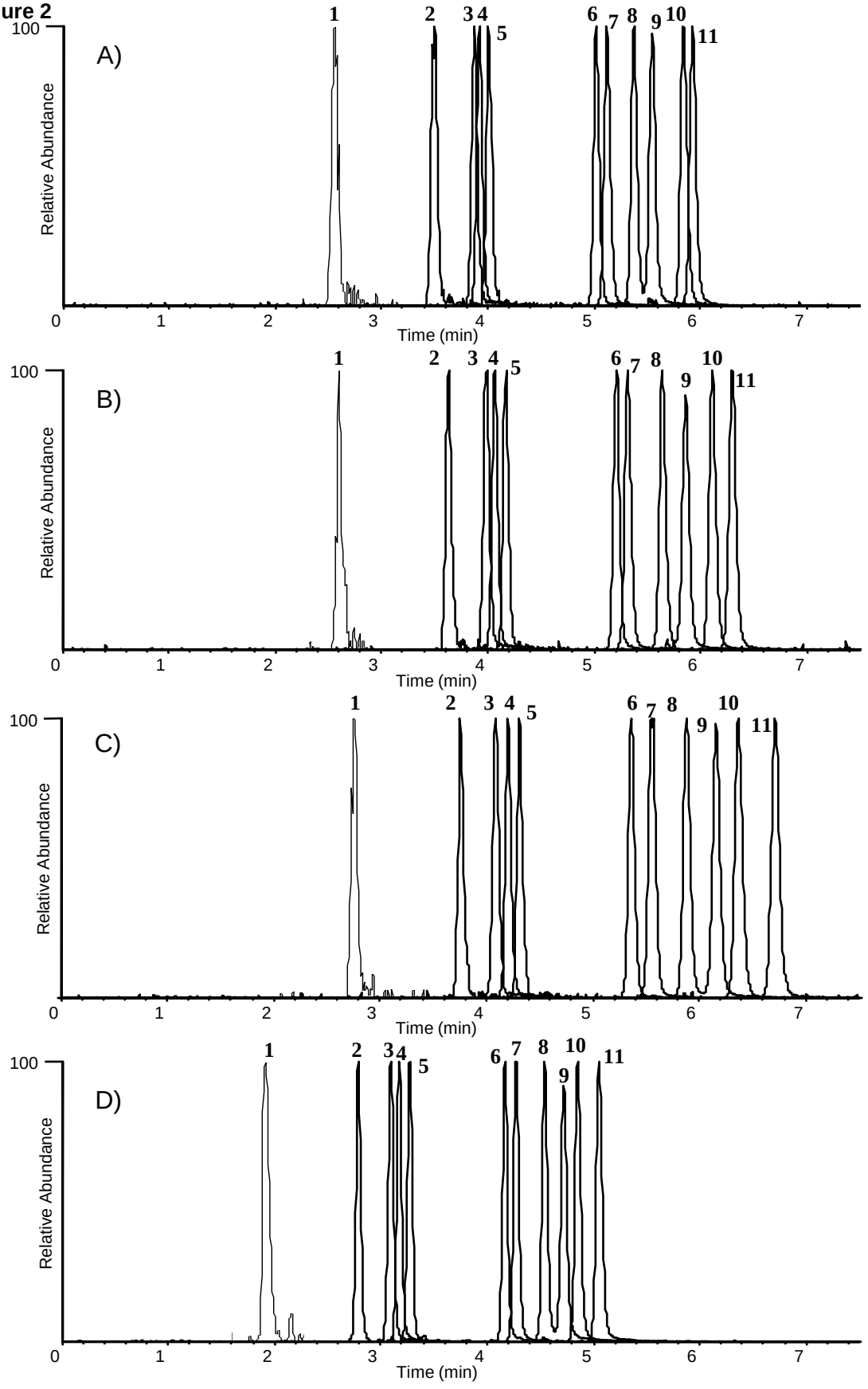


Figure 2

Figure 3

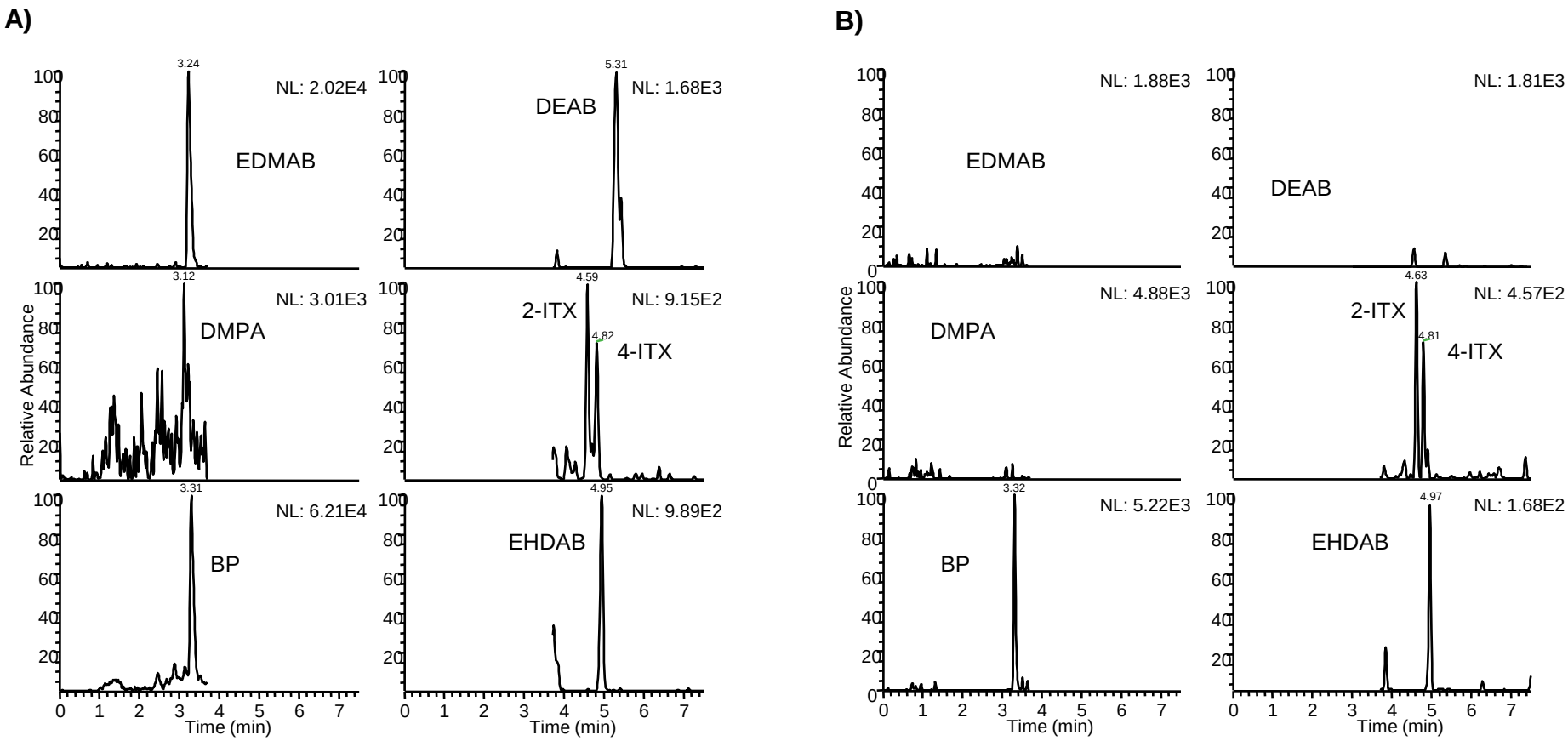

A)
B)
EDMAB
DEAB
DMPA
2-ITX
4-ITX
BP
EHDAB
NL: 2.02E4
NL: 1.68E3
NL: 3.01E3
NL: 9.15E2
NL: 6.21E4
NL: 9.89E2
NL: 1.88E3
NL: 1.81E3
NL: 4.88E3
NL: 4.57E2
NL: 5.22E3
NL: 1.68E2
3.24
5.31
3.12
4.59
4.82
3.31
4.95
4.63
4.81
3.32
4.97
Relative Abundance
Time (min)

Table 1

Table 1. SRM acquisition parameters

Segment	Time (min)	Analyte	Precursor ions	Product ion Assigment (Quantifier/Qualifier)	Collision energy (CE, V)	Ion Ratio (%RSD)
1	0-3.7	HMPP	165.1 $[M+H]^+$	91.1 $[C_7H_7]^+$ 119.0 $[M+H-H_2O-C_2H_4]^+$	11 23	1.1 (10)
		HCPK	205.1 $[M+H]^+$	105.0 $[C_7H_5O]^+$ 187.1 $[M+H-H_2O]^+$	13 5	2.6 (9)
		EDMAB	194.1 $[M+H]^+$	151.1 $[M+H-CH_3-C_2H_4]^{+\bullet}$ 134.1 $[M+H-CH_3-C_2H_5O]^+$	23 31	1.4 (2)
		DMPA	225.1 $[M-CH_3O]^+$	197.1 $[M-CH_3O-CO]^+$ 105.0 $[C_7H_5O]^+$	14 23	1.8 (10)
		BP	183.1 $[M+H]^+$	105.0 $[C_7H_5O]^+$ 77.0 $[C_6H_5]^+$	15 34	1.3 (8)
2	3.7-6.0	PBZ	259.1 $[M+H]^+$	105.0 $[C_7H_5O]^+$ 181.1 $[M+H-C_6H_6]^+$	17 18	2.7 (2)
		DEAB	325.2 $[M+H]^+$	176.1 $[M+H-C_{10}H_{15}N]^+$ 281.2 $[M+H-C_2H_5-CH_3]^+$	28 27	2.6 (3)
		2-ITX / 4-ITX	255.1 $[M+H]^+$	213.0 $[M+H-C_3H_6]^+$ 184.0 $[M+H-C_3H_6-CHO]^{+\bullet}$	22 40	1.9 (4)
		2-ITX-D7	262.1 $[M+H]^+$	214.0 $[M+H-C_3D_6]^+$ 185.0 $[M+H-C_3D_6-CHO]^{+\bullet}$	23 42	1.8 (5)
		DETX	269.1 $[M+H]^+$	241.1 $[M+H-C_2H_4]^+$ 213.0 $[M+H-C_2H_4-C_2H_4]^+$	23 30	1.1 (3)
		EHDAB	278.2 $[M+H]^+$	151.1 $[M+H-CH_3-C_8H_{16}]^{+\bullet}$ 134.0 $[M+H-CH_3-C_8H_{17}O]^+$	23 27	4.4 (4)

Table 2. Comparison of SPE and QuEChERS extraction procedures using a baby food sample matrix.

Compound	SRM ILOQ (pg)	SPE method				QuEChERS method			
		MLOQ (µg/kg)	Trueness (%)**	run-to-run precision**	day-to-day precision**	MLOQ (µg/kg)	Trueness (%)**	run-to-run precision**	day-to-day precision**
HMPP	12000	710	91	2.7	6.5	666	94	2.9	7.2
HCPK	600	500	89	1.9	7.6	500	87	2.6	7.8
EDMAB	30	0.5	90	2.8	6.8	0.5	81	4.5	8.6
DMPA	300	1.5	88	2.1	7.2	0.7	83	3.4	7.1
BP	300	2.0	92	4.3	8.6	2.3	97	5.1	9.7
PBZ	30	0.7	91	5.1	9.2	0.7	88	4.6	8.9
DEAB	15	0.3	89	4.9	9.8	0.7	98	5.0	10.1
2-ITX	1.5	0.2	90	3.3	6.4	0.2	93	3.3	7.1
4-ITX	1.5	0.2	92	2.7	6.8	0.2	95	3.4	6.7
DETX	1.5	0.3	91	3.3	7.2	0.3	95	4.3	7.6
EHDAB	15	0.7	90	4.2	8.3	1.0	86	4.4	8.9

*Injection volume: 10 µL

**Spiked concentrations (µg L^{-1}): HMPP (2530), HCBPK (800), EDMAB (0.3), DMPA (4), BP (80), PBZ (1.4), DEAB (0.3), 2-ITX (0.14), 4-ITX (0.14), DETX (0.14) and EHDAB (0.3)

Table 3

Table 3. SRM vs H-SRM (Q1) in a pineapple juice and a baby food matrices.

Compound	Pineapple matrix				Baby food matrix			
	SRM		H-SRM (Q1)		SRM		H-SRM (Q1)	
	Peak Signal (%)	S/N ratio	Peak Signal (%)	S/N ratio	Peak Signal (%)	S/N ratio	Peak Signal (%)	S/N ratio
HMPP	100	12	44	20	100	15	51	100
HCPK	100	14	63	30	100	15	62	30
EDMAB	100	40	48	50	100	20	57	25
DMPA	100	30	45	60	100	20	50	100
BP	100	70	43	500	100	60	41	450
PBZ	100	10	25	300	100	10	26	110
DEAB	100	210	25	300	100	130	26	250
2-ITX	100	250	27	750	100	200	27	500
4-ITX	100	250	30	900	100	260	29	700
DETX	100	40	30	800	100	20	30	300
EHDAB	100	150	30	250	100	60	37	200

Table 4

Table 4. Packaged food samples analyzed using QuEChERS LC-MS/MS method using H-SRM (μg kg^{-1}).

Sample type	Packaging volume (mL)	HMPP	HCPK	EDMAB	DMPA	BP	PBZ	DEAB	2-ITX	4-ITX	DETX	EHDAB
Baby food 1 (fruit and cereal)	250	n.d.	n.d.	~MLOD	n.d.	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Baby food 2 (milk and cereal)	250	n.d.	n.d.	n.d.	n.d.	29	n.d.	n.d.	n.d.	n.d.	n.d.	~MLOD
Baby food 3 (milk, fruit, cereal)	250	n.d.	n.d.	~MLOD	~MLOD	n.c.*	n.d.	n.d.	0.8	n.d.	~MLOD	0.6
Baby food 4 (multi-fruit)	200	n.d.	n.d.	0.5	n.d.	3.0	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
Fruit juice 1 (peach and grape)	200	n.d.	n.d.	n.d	n.d.	2.5	n.d.	n.d.	0.2	~MLOD	0.07	n.d.
Fruit juice 2 (orange)	200	n.d.	n.d.	n.d	n.d.	6.5	n.d.	n.d.	0.2	~MLOD	n.d.	0.6
Fruit juice 3 (pineapple)	200	n.d.	n.d.	n.d.	n.d.	2.8	n.d.	0.7	0.2	0.07	n.d.	n.d.
Gazpacho 1	1000	n.d.	n.d.	2.5	n.d.	n.c.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gazpacho 2	1000	n.d.	n.d.	0.5	n.d.	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gazpacho 3	1000	n.d.	n.d.	1.6	n.d.	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gazpacho 4	1000	n.d.	n.d.	0.5	n.d.	8.0	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
White wine	1000	n.d.	n.d.	n.d.	n.d.	1.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sangria	1000	n.d.	n.d.	n.d.	n.d.	1.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Water	1000	n.d.	n.d.	n.d	n.d.	3.8	n.d.	n.d.	~MLOD	n.d.	n.d.	n.d.

n.d.: not detected.

*n.c.: not confirmed. Ion ratio error higher than 20%.