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# Improving the monitoring of multi-class pesticides in baby foods using QuEChERS-UHPLC-Q-TOF with automated identification based on MS/MS similarity algorithms

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1 **Improving the monitoring of multi-class pesticides in baby foods**  
2 **using QuEChERS-UHPLC-Q-TOF with automated identification**  
3 **based on MS/MS similarity algorithms**

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## 33 **Abstract**

34 A screening method was developed for the multi-residue analysis of pesticides in baby foods  
35 using QuEChERS and UHPLC-Q-TOF. For sample preparation, the two-buffered versions of  
36 QuEChERS and different purification procedures were studied. False negatives and false  
37 positives were determined using different thresholds mentioned in the literature on the  
38 retention time and accurate mass measurement detection criteria. To reach unequivocal  
39 identification, the fragmentation spectra of the pesticides were used. The information-  
40 dependant-acquisition (IDA) mode was optimized with a precursor-inclusion list (PIL) to  
41 limit the loss of MS/MS data. Then, the experimental fragmentation spectra were compared to  
42 those included in a homemade library, by assessing different MS/MS algorithms and  
43 similarity scores. The optimised method was validated according to SANTE/11312/2021  
44 guidelines. 95% and 73% of the pesticides presented a screening detection limit (SDL) and a  
45 limit of identification (LOI)  $\leq 0.1 \text{ mg.kg}^{-1}$ . One plasticizer was found in the investigated  
46 samples by a suspect-screening approach.

## 47 **Keywords**

48 LC-HRMS; Screening method; Acquisition mode; Detection; Identification; Method  
49 validation

## 50 **1. Introduction**

51 In 2005, C. Wild introduced the concept of exposome as the total environmental and non-  
52 genetic exposure sources and factors that an organism undergoes throughout their lifetime,  
53 potentially associated with multifactorial disorders (Wild, 2005). In the context of dietary  
54 exposure, non-genetic factors to which humans can be exposed include microbiological  
55 agents and chemicals, such as pesticides. Pesticides represent a wide-range of bioactive  
56 chemical compounds of low molecular weight, intensively used in modern agriculture to  
57 control pest organisms and increase crop yields. During the past few decades, their  
58 widespread use has caused serious concerns worldwide regarding food quality and safety  
59 aspects. Importantly, their application to crops and use in livestock may result in chemical  
60 pollution in food products (Pang et al., 2021), which exposes the population to a wide array of  
61 pesticides occurring in a dynamic range of concentrations. Another important aspect is that  
62 exposure to mixtures of pesticide residues at different levels may enhance their harmfulness  
63 or, on the contrary, reduce it through potentiating effects and synergistic agonistic or  
64 antagonistic interactions, also known as "cocktail effects" (Lukowicz et al., 2018; Rizzati et  
65 al., 2016). As a result, due to their known or potential adverse effects on human health  
66 (Ntzani et al., 2013), there is a need to assess the risks of chronic exposure to pesticides  
67 through diet.

68 The risks to health are even higher in infants below 3 years of age, who represent a more  
69 vulnerable group (Nougadère et al., 2020; Aguilera-Luiz et al., 2012). In Europe, the  
70 European Food Safety Authority (EFSA) has set maximum residue levels (MRLs) of  
71 individual pesticide residues in common animal and plant-based food products for human  
72 consumption (European Commission, 2005). Baby foods, a term used to designate mixtures of  
73 different matrices formulated for infants, including fruits and vegetables, animal and cereal-  
74 based food products (Hercegová et al., 2007), follow a standardised MRL of 0.01 mg.kg<sup>-1</sup>  
75 (European Commission, 2006).

76 In recent years, multi-residue screening of pesticides has developed significantly with the  
77 introduction of new methods, especially regarding sample preparation. The Quick, Easy,  
78 Cheap, Efficient, Rugged and Safe (QuEChERS) procedure stands out as one of the most  
79 commonly used methods for extraction of multi-class pesticides in food samples (González-  
80 Curbelo et al., 2015), including baby foods (Pérez-Ortega et al., 2017; Jia et al., 2014;  
81 Aguilera-Luiz et al., 2012). The QuEChERS procedure involves extraction using acetonitrile

82 and partitioning salts, followed by a clean-up step (Anastassiades et al., 2003). Although the  
83 QuEChERS procedure is well suited to extraction of multi-class pesticides with high yields in  
84 various matrices, there is no appropriate one-size-fits-all method. Therefore, prior  
85 optimisation may be required to improve extraction efficiency.

86 Regarding analytical techniques, gas and reversed-phase liquid chromatography coupled with  
87 tandem mass spectrometry (LC/GC-MS/MS) operating in multiple reaction monitoring  
88 (MRM) mode are the most developed technique for routine pesticide determination  
89 (Villaverde et al., 2016; Alder et al., 2006). Although these methods are very sensitive and  
90 robust, a major limitation is that they are limited to a predetermined list of targeted  
91 compounds to be searched *a priori*. These targeted approaches are blind to any compound  
92 outside this list.

93 The rise of high-resolution mass spectrometry (HRMS) has made it possible to overcome this  
94 limitation. Unlimited determination of compounds without a pre-defined list has become  
95 possible using high-resolution mass spectrometers such as the time-of-flight (TOF) or  
96 Orbitrap instruments operating in full-scan MS mode (Weng et al., 2020). This feature  
97 improved the concept of screening methods and allowed for the development of suspect and  
98 non-targeted screening approaches, which extend the analytical scope (Dubocq et al., 2021;  
99 Phillips et al., 2018). Furthermore, retrospective analysis of acquired full-MS data is possible  
100 when a new contaminant was not characterised at the time of injection (Gómez-Pérez et al.,  
101 2015).

102 Detection of pesticides in food using HRMS operating in full-scan mode is based on the  
103 retention time ( $t_r$ ) and accurate mass measurement of a precursor ion. To date, there is no  
104 established consensus on the thresholds to be used for these criteria, even though analytical  
105 guidelines (SANTE, 2021; FDA, 2015) provide various recommendations. For instance,  
106 SANTE guidelines recommend a  $t_r$  tolerance of  $\pm 0.1$  min and accurate mass measurement  $\pm$   
107 5 ppm for screening of pesticides in food, whereas some authors prefer to use wider  
108 thresholds (Wang et al., 2019; Pérez-Ortega et al., 2017; Luz Gómez-Pérez et al., 2015). This  
109 is a crucial parameter to consider as the data processing parameters may influence the balance  
110 between false negatives and false positives (López et al., 2014). Given this consideration, an  
111 assessment on the thresholds to be used should be carried out at the time of method  
112 development, to provide for efficient and reliable automated-based data processing, which is a  
113 key for implementation of HRMS in routine use.

114 To reach a higher degree of confidence and limit the occurrence of false positives, screening  
115 of pesticides may also include MS/MS data for unequivocal identification ([Schymanski et al.,](#)  
116 [2014](#)). In HRMS, full-scan MS mode is often followed by MS/MS data acquisition in the  
117 same run-analysis, which can be performed in two ways: full-scan mode followed by either  
118 information-dependent-acquisition (IDA) or data-independent acquisition (DIA). IDA mode  
119 provides MS/MS spectra of high quality, but all the analytes of interest are not systematically  
120 fragmented ([Guo et al., 2020](#)). To overcome this limitation, a precursor-inclusion list (PIL)  
121 can be included to prioritize the compounds to be fragmented in targeted analysis. Another  
122 option is the use of DIA mode, in which all the precursor ions are fragmented, providing more  
123 complex spectra ([Diallo et al., 2022](#)). There are no requirements on the mode to be used for  
124 identification. In guidelines ([SANTE, 2021](#); [FDA, 2015](#)), identification entails the use of  $t_r$   
125 and accurate mass measurement of a precursor ion and corresponding product ion(s).  
126 However, not all instrumental software supplies a mass error (ppm) on product ions, and the  
127 identification then relies on the similarity of the whole MS/MS spectrum of a compound,  
128 against its reference spectrum included in a home-made library. The degree of similarity is  
129 displayed by scores (%) measured using MS/MS search algorithms. Nevertheless, this  
130 approach is not covered by the guidelines, which may be detrimental if this is the only option  
131 provided by the software. In the literature, few studies have reported the use of automated  
132 identification based on MS/MS spectra similarity for multi-residue screening of pesticides in  
133 food ([Wang et al., 2016](#)).

134 The aim of this work was to develop a simple and reliable method for the screening of 204  
135 pesticides in baby food samples using UHPLC-Q-TOF analysis. The sample preparation  
136 conditions were optimised based on a QuEChERS protocol that can be used for baby food of  
137 heterogeneous and varied composition. To the best of our knowledge, this study is the first  
138 evaluating different QuEChERS methods and clean-up procedures to analyse multi-class  
139 pesticides in such heterogeneous matrices as baby foods. False negatives and false positives  
140 were determined using different thresholds mentioned in the literature on the  $t_r$  and accurate  
141 mass measurement criteria.. Another part of the work focused on the identification of the  
142 pesticides. Thus, the impact of a PIL during data acquisition was evaluated on the  
143 performances of identification. Furthermore, this study is the first to evaluate the impact of  
144 different MS/MS search algorithms and similarity scores, regarding the performances of  
145 identification of multi-class pesticides in baby food samples. The method was validated based  
146 on the SANTE guidelines ([SANTE, 2021](#)). The screening detection limit (SDL) of each of the

147 studied pesticides was thus established. Although there is no requirement for meeting  
148 identification criteria, the limit of identification (LOI) was also considered as a validation  
149 parameter. The versatility of the method was tested using an expanded list of organic  
150 contaminants in suspect-screening analysis.

151

## 152 **2. Material and Methods**

### 153 *2.1. Chemicals and reagents*

154 A kit of 204 LC pesticide standards, divided into ten mixtures (100 mg.L<sup>-1</sup> of each compound  
155 in either acetonitrile (MeCN) or methanol (MeOH)), was obtained from Restek Corporation  
156 (Ulis, France). HPLC–MS-grade MeCN and formic acid (FA) were obtained from  
157 Thermofisher Scientific Corporation (Illkirch, France). Ultrapure water (18.2 MΩ.cm) used  
158 for reagent and sample preparation was obtained through a Millipak 40 Gamma Gold Filter  
159 (pore size 0.22 μm) and using a Milli-Q Ultrapure and Pure water system purchased from  
160 Merck Millipore (Saint-Quentin-en-Yvelines, France). A QuEChERS extraction kit, based on  
161 the AOAC Method and containing 4.0 g of magnesium sulphate (MgSO<sub>4</sub>) and 1.0 g of sodium  
162 acetate, was purchased from Agilent (Santa Clara, CA, USA). A QuEChERS extraction kit  
163 based on EN Method 15662 and containing 4.0 g of MgSO<sub>4</sub>, 1.0 g of sodium chloride (NaCl),  
164 1.0 g of sodium citrate and 0.5 g of sodium citrate sesquihydrate, was purchased from Agilent  
165 (Santa Clara, CA, USA). A Dispersive solid phase extraction (d-SPE) kit containing 150 mg  
166 of primary and secondary amines (PSA) and 900 mg of MgSO<sub>4</sub> was purchased from Agilent  
167 (Santa Clara, CA, USA). A kit of d-SPE containing 400 mg of PSA, 1200 mg of MgSO<sub>4</sub>, 400  
168 mg of octadecylsilyl silica (C18) and 45 mg of Graphitized Carbon Black (GCB) was  
169 obtained from Agilent (Santa Clara, CA, USA). An SPE Oasis Prime HLB purification  
170 cartridge was acquired from Waters (Milford, MA, USA).

171

### 172 *2.2. Equipment and apparatus*

173 IKA Ultra-Turrax T25 mixer used for sample extraction was obtained from Retsch  
174 Technology GmbH (Haa, Germany). A Genie 2 vortex used for sample extraction procedure  
175 was purchased from Scientific Industries (Bohemia, USA), and an Eppendorf 5810 centrifuge  
176 was purchased from Eppendorf (Hamburg, Germany).

177 The UHPLC-Q-TOF system used for analysis consisted of a Dionex UltiMate-3000 system  
178 with a binary pump and autosampler (ThermoFisher Scientific, Waltham, USA), coupled with  
179 a Q-TOF mass spectrometer (TripleTOF 5600<sup>TM</sup>, AB Sciex, Darmstadt, Germany) equipped  
180 with an electrospray ionization source (ESI).

181

### 182 2.3. *Preparation of standard solutions*

183 The ten different mixtures (#1 to #10), 100 mg.L<sup>-1</sup>, were transferred and stored in screw-  
184 capped amber glass tubes at -20°C. There were 13 pesticides in mix #1, 16 pesticides in #2,  
185 38 in #3, 63 in #4, 30 in #5, 28 in #6, 7 in #7, 1 in #8, 7 in #9, and finally 1 pesticide in mix  
186 #10. A mixture of the 204 pesticides (2 mg.L<sup>-1</sup>) was prepared by a suitable dilution of the 10  
187 individual mixtures with MeCN, and stored in screw-capped amber glass tubes at -20°C. The  
188 composition of each mix is supplied in electronic supplementary materials (ESM) (Table S1).

### 189 2.4. *Pesticides selected*

190 The 204 pesticides included in this study were selected in view of their worldwide occurrence  
191 in food samples. The wide range of physicochemical properties of these pesticides make them  
192 a relevant model for a multi-residue method. Among these compounds, only the 63 pesticides  
193 belonging to mix #4 (Table S1) were selected for the fortification of baby food in the sample  
194 preparation optimisation-stage. They were selected as they cover the dynamic range of  
195 polarity (LogP) of the rest of the studied pesticides. The complete mixture of 204 pesticides  
196 was used for the fortification of the baby food samples for the following parts: (i) impact of  
197 different criteria thresholds on the performances of the method, (ii) impact of a PIL and  
198 different MS/MS algorithms on the performances of the method, and (iii) method validation.

199

### 200 2.5. *Matrices selected*

201 Fourteen baby food commodities of various matrix compositions were purchased from local  
202 markets (Maisons-Alfort, France), and stored in a freezer at -20 °C. The composition of each  
203 baby food is supplied in ESM (Table S2). Four of the fourteen baby food samples (#1 to #4),  
204 representative of the remaining foods in terms of matrix composition, were selected for  
205 optimisation of sample preparation. The ten remaining baby food samples (#5 to #14) were  
206 used for the other parts of the study: (i) impact of different criteria thresholds on the  
207 performances of detection, (ii) impact of a PIL and different MS/MS algorithms on the  
208 performances of identification, (iii) method validation, and (iv) suspect screening analysis.



209

## 210 2.6. UHPLC-Q-TOF analysis

211 Reversed-phase gradient liquid chromatography separation was performed using an  
212 Aqua<sup>®</sup> C18 column (150 x 2 mm, 3- $\mu$ m particle size, 125 Å pore diameter; Phenomenex Inc,  
213 USA) equipped with an Aqua<sup>®</sup> pre-column (4.0 mm x 2.0 mm, 3- $\mu$ m particle size,  
214 Phenomenex Inc, USA). The column temperature was thermostatted at 50°C. The mobile  
215 phase composition consisted of ultrapure water (eluent A) and MeCN (eluent B), both  
216 containing 0.01% of FA. The gradient profile was started with 10% B (0 min); increased  
217 linearly to 100% B for 9 min (0-9 min); fixed for 3 min (9–12 min), returned to the initial  
218 conditions for 0.1 min (12.1 min), and then followed by a re-equilibration step for 3.9 min.  
219 The total run time was 16 min. The autosampler was thermo-regulated at 15°C. The injection  
220 volume was 10  $\mu$ L and the gradient flow rate was constant and set at 0.5 mL.min<sup>-1</sup>.

221

222 Before each batch, the TripleTOF 5600<sup>TM</sup> system was systematically tuned and calibrated  
223 manually using the APCI positive calibration solution (AB Sciex technology) in TOF MS and  
224 TOF MS/MS in high-resolution mode. Furthermore, the automated calibration device system  
225 (CDS) was scheduled to perform automatic calibration every five samples during the run-  
226 analysis. ESI+ parameters were as follows: ion spray voltage floating (ISVF) was set at 5.5  
227 kV, source temperature (TEM) 500°C, curtain gas (CUR) 30 psi, nebulising gas (GS1) 40 psi,  
228 and heater gas (GS2) 45 psi. In this study, two methods based on IDA mode were carried out  
229 using the UHPLC-Q-TOF system:

230 – **Method A** was a combination of full scan TOF-MS / IDA mode. Five experiments were  
231 conducted in a single-run analysis. The first experiment was a full-scan TOF-MS acquisition  
232 that covered a mass range of 50-1000 Da. The declustering potential was set at 80 eV and the  
233 collision energy (CE) was set at 10 eV. The accumulation time was set at 490 ms. The  
234 experiments 2-5 consisted in MS/MS data acquisition using IDA mode. The accumulation  
235 time was set at 100 ms for each IDA experiment, and the CE was set at 40 eV with a spread of  
236  $\pm$  20 eV (20, 40 and 60 eV) in high-resolution mode. The collision-induced dissociation gas  
237 used was nitrogen. Dynamic background subtraction was turned on. The total cycle time was  
238 about 900 ms. Considering that the chromatographic peak width was approximately 15 s,  
239 the obtained number of points per peak was about 16-17 which is quite enough according to  
240 the SANTE guidelines.

241

242 – **Method B** consisted in the same full scan TOF-MS / IDA acquisition mode than  
243 Method A, with the same parameters. However, in this method, a PIL of the 204-targeted  
244 pesticides was implemented to avoid the possible non-fragmentation of analytes. PIL  
245 comprised the 204 precursor ion masses in forms of  $[M+H]^+$ ,  $[M+Na]^+$ ,  $[M+NH_4]^+$ , or in-  
246 source fragments, as well as their corresponding chromatographic  $t_r$ . These informations are  
247 supplied in [ESM \(Table S1\)](#).

248  
249 Data acquisition was performed using Analyst<sup>®</sup> software (version 1.7) of AB Sciex (Les Ulis,  
250 France). The resulting extracted ion chromatograms (XICs) were reconstructed using  
251 PeakView<sup>®</sup> software, version 2.2 comprising the MasterView<sup>™</sup> module of AB Sciex.

252

### 253 2.7. *Development of a homemade MS/MS library*

254 A homemade MS/MS library of 204 pesticides was built using the convenient LibraryView<sup>™</sup>  
255 software, version 1.0.3 of AB Sciex. The library was built by injecting separately each of the  
256 ten mixtures diluted to  
257 0.2 mg.L<sup>-1</sup> in MeCN. Exact mass, the CAS number and the MS/MS fragmentation spectrum  
258 of each of the studied pesticides were recorded. The fragmentation spectra included in the  
259 homemade library are considered as reference spectra.

260

### 261 2.8. *Optimisation of sample preparation*

262 Optimisation of extraction was performed comparing the two-buffered variants of  
263 QuEChERS, the AOAC 2007.01 ([Lehotay, 2007](#)) and CEN 15662 ([Anastassiades et al., 2007](#))  
264 methods. In this study, these methods were modified including a hydrating strategy and  
265 without extraction solvent acidification.

266 Sample preparation optimisation is presented in [Fig. 1](#). Before use, the samples were thawed  
267 at 4°C overnight. No sample pre-preparation was required. For the enrichment experiment, 15  
268 µL of mix #4 diluted to 2 mg.L<sup>-1</sup> was added to 50 mL-polypropylene centrifuge tubes, which  
269 contained 3.0 g of blank sample ± 0.2 g, to provide a spiking level at 0.01 mg.kg<sup>-1</sup> of  
270 pesticides. A volume of 7 mL of ultrapure water was added to each tube, and then ground  
271 using an IKA Ultra-Turrax mixer for 3 min at 5000 rpm. Then, 10 mL of MeCN was added to  
272 each centrifuge tube to perform the extraction of pesticides, which were vortexed again for 1  
273 min. Then, either AOAC or CEN extraction salts were added to the fortified samples, and the  
274 resulting mixture was centrifuged for 5 min at 4000 rpm. 1 mL of the resulted supernatant was

275 deposited into a 2 mL-amber vial for injection in the UHPLC-Q-TOF system operating with  
276 **Method A.**

277 Sample preparation optimisation also included a comparison of different protocols of  
278 purification after the extraction-stage, in addition to the protocol without purification (Fig. 1).  
279 When a purification step was performed, 6 mL of the supernatant was recovered and  
280 deposited/percolated into either:

- 281 - A 15-mL centrifuge tube containing 900 mg of MgSO<sub>4</sub> and 150 mg of PSA;
- 282 - A 15-mL centrifuge tube containing 1200 mg of MgSO<sub>4</sub>, 400 mg of PSA, 400 mg of  
283 C18 and 45 mg of GCB;
- 284 - An Oasis HLB cartridge, without prior conditioning.

285

286 Finally, 1 mL of the supernatant was deposited into a 2 mL-amber vial prior to injection in the  
287 UHPLC-Q-TOF system operating with **Method A.**

288 All protocols (Fig. 1) were performed in triplicate. The assessment was carried out  
289 considering the false negative rates of each procedure. A pesticide was considered a false  
290 negative when a pesticide did not fit a  $t_r$  variation  $\pm 0.1$  min and/or accurate mass  
291 measurement  $\pm 5$  pm (SANTE, 2021).

292

### 293 2.9. *Impact of different detection criteria thresholds on the performances of* 294 *detection*

295 In this section, the term “detection” was used to define the screening of pesticides using  $t_r$  and  
296 accurate mass measurement criteria on the precursor ions. In this study, an assessment of the  
297 criteria of detection was carried out using the following thresholds: (i)  $t_r$  variation  $\pm 0.1$  min,  
298 accurate mass measurement  $\pm 5$  ppm (SANTE, 2021); (ii)  $t_r$  variation  $\pm 0.5$  min, accurate  
299 mass measurement  $\pm 5$  ppm (Wang et al., 2019; FDA, 2015); (iii)  $t_r$  variation  $\pm 0.25$  min,  
300 accurate mass measurement  $\pm 10$  ppm (Pérez-Ortega et al., 2017).

301 Each of the studied thresholds was evaluated regarding the false negative and false positive  
302 rates obtained. A false negative was defined when a pesticide did not fit the expected  
303 thresholds for  $t_r$  and/or mass measurement criteria. In contrast, a false positive was defined  
304 when the software reported a positive hit, i.e., when a pesticide fitted both  $t_r$  and accurate  
305 mass measurement criteria thresholds, in the non-spiked samples. The same list of 204  
306 pesticides was used for the calculation of false negatives and false positives.

307 2.10. *Impact of a precursor ion list and different MS/MS similarity algorithms on the*  
308 *performances of identification.*

309  
310 In this section, the term “identification” was used to define the screening of the 204 pesticides  
311 using  $t_r$  variation  $\pm 0.1$  min and accurate mass measurement  $\pm 5$  ppm on the precursor ion, as  
312 well as the characteristic MS/MS fragmentation spectra. In this part of the work, the baby  
313 food samples were acquired using Method A and Method B, in order to evaluate the impact of  
314 the PIL on the performances. Then, the generated files were processed using MasterView™  
315 software. The experimental fragmentation spectra obtained were compared to those included  
316 in the homemade library. This comparison was automatically computed by the software using  
317 MS/MS search algorithms. Three algorithms are available in the software, and were assessed  
318 in this study:

- 319 - The *purity algorithm* (PUR) measures the similarity between the experimental and  
320 reference spectra. All fragment ions from both spectra and their relative abundance are  
321 used for calculation;
- 322 - The *fit algorithm* (FIT) measures the degree to which the library spectrum is contained  
323 in the experimental spectrum, disregarding additional fragment ions present in the  
324 experimental spectrum but absent in the reference spectrum. All the expected fragment  
325 ions and their relative abundance are used for calculation;
- 326 - The *Reverse Fit* algorithm (RFIT) measures the degree to which the experimental  
327 spectrum is contained in the library spectrum, disregarding the fragment ions present  
328 in the library spectrum but absent in the experimental spectrum. All the experimental  
329 fragment ions and their relative abundance are used for calculation.

330 These algorithms automatically calculate a degree of confidence characterised by a similarity  
331 score (%), allowing for fast and reliable identification, as illustrated in [ESM \(Fig. S1\)](#). Two  
332 similarity scores: (i) >50% and (ii) >70%, used elsewhere ([Ogawa et al., 2020](#); [Wang et al.,](#)  
333 [2016](#)), were considered as the thresholds to be fulfilled. A pesticide that was not fitting the  
334 above mentioned criteria or not fragmented was reported a false negative.

335

336 2.11. *Method validation*

337 The method was validated according to the SANTE guidelines for screening methods. Method  
338 validation was conducted at 0.01 and 0.1 mg.kg<sup>-1</sup> to establish the screening detection limits  
339 (SDL) (SANTE, 2021) and the limits of identification (LOI). Experiments were carried out in  
340 triplicate for repeatability assessment. Each replicate was injected in a single batch, on three  
341 different days, to include inter-batch and instrumental over-time variabilities.. The SDL was  
342 established as the lowest concentration for which a compound was detected following (i) t<sub>r</sub>  
343 variation ± 0.1 min and (ii) accurate mass measurement ± 5 ppm, in at least 95% of the  
344 samples (SANTE, 2021), i.e., in at least 29 out of the 30 baby food samples. In addition, the  
345 LOI was established as the lowest concentration for which a pesticide was correctly identified  
346 in at least 95% of the samples, i.e., in at least 29 out of the 30 baby food samples. The LOI  
347 used the same criteria as for the SDL, plus a comparison between experimental and reference  
348 fragmentation spectra using FIT algorithm with a similarity score >70%.

349

### 350 **3. Results and discussion**

#### 351 *3.1. Sample preparation optimisation*

352 The sample preparation was studied and optimised regarding different extraction and purification  
353 procedures as explained in Section 2.7. The results for the four baby foods are displayed in [Fig.](#)  
354 [2](#).

355

356 When using AOAC mixture salts, false negative rates ranged from 22–32%, 19–33%, 21–32%  
357 and 13–22% in baby food 1 to 4, respectively regardless of the purification procedure used.  
358 Then, when using CEN mixture salts, false negative rates varied from 29–38%, 32–39%, 29–  
359 38% and 21–29% in baby food 1 to 4, respectively regardless of the purification procedure used.  
360 According to the results, the extraction of pesticides appeared to be more effective using AOAC  
361 salts. Regarding the purification following AOAC-based extraction, false negatives varied from  
362 19–27%, 13–22%, 22–33% and 19–32% using no purification, MgSO<sub>4</sub>/PSA,  
363 MgSO<sub>4</sub>/PSA/C18/GCB and the Oasis PRIME HLB cartridge, respectively. Regarding the  
364 purification following CEN-based extraction, false negatives varied from 23–34%, 21–32%, 21–  
365 36% and 29–39% using no purification, MgSO<sub>4</sub>/PSA, MgSO<sub>4</sub>/PSA/C18/GCB and the Oasis  
366 PRIME HLB cartridge, respectively. According to the results, purification using MgSO<sub>4</sub>/PSA  
367 was the most effective to clear the extracts achieved with the AOAC or CEN methods.

368

369 The results obtained separately from the four baby foods (Fig. 2) were grouped together to  
370 increase the statistical power and to evaluate the overall performances, irrespective of the matrix  
371 composition. The results are displayed in [ESM \(Fig. S2\)](#). Average false negative rates ranged  
372 from 19–30% for the AOAC method against 28–36% for the CEN method, regardless of the  
373 purification procedure. Performances of detection were better when using the AOAC method,  
374 which was statistically confirmed (two-way ANOVA:  $p$ -value < 0.01). Therefore, the acetate-  
375 buffered version of QuEChERS was retained for the extraction of pesticides. This was consistent  
376 with other studies in which AOAC mixture salts were used for screening of multi-class  
377 contaminants in baby foods ([Pérez-Ortega et al., 2017](#); [Aguilera-Luiz et al., 2012](#)).

378  
379 Then, the four purification protocols were assessed for the retained AOAC method. On average,  
380 false negative rates were 23%, 19%, 30% and 27% when using the procedure without  
381 purification, MgSO<sub>4</sub>/PSA, MgSO<sub>4</sub>/PSA/C18/GCB and the Oasis PRIME HLB cartridge,  
382 respectively. Standard deviations (SD) were equivalent overall. Purification using MgSO<sub>4</sub>/PSA  
383 appeared to be the most efficient protocol to remove matrix interferences and improve compound  
384 detectability. MgSO<sub>4</sub> acts as a desiccant as it dries out the water to improve the partitioning  
385 process ([Li et al., 2021](#)), whereas PSA is commonly used in various food as a sorbent to remove  
386 co-extractives sugars, organic acids, fatty acids and certain pigments ([Walorczyk et al., 2015](#)).  
387 Nevertheless, there was no significant difference between this protocol and the protocol without  
388 a purification step. Thus, to be able to select the most suitable protocol (MgSO<sub>4</sub>/PSA vs no  
389 purification), the signal-to-noise ratio (S/N ratio) was considered. The results are supplied in  
390 [ESM \(Fig. S3\)](#). The average S/N ratios were 371 and 476 without purification and with  
391 MgSO<sub>4</sub>/PSA, respectively. On average, pesticide signals were better when using a clean-up step  
392 with MgSO<sub>4</sub>/PSA, which was statistically confirmed (t-test,  $p$ -value < 0.05). Purification using  
393 MgSO<sub>4</sub>/PSA was the most efficient protocol to [improve the S/N of the pesticides and](#) was  
394 therefore retained for the method. The lower S/N ratio in the samples without purification can be  
395 explained by the higher amount of co-extracted compounds (sugars, organic acids, fatty acids,  
396 pigments, etc.), which could lead to signal suppression of pesticides during the ionisation process  
397 ([Petrarca et al., 2022](#)). The use of MgSO<sub>4</sub>/PSA as a purification method was also conducted by  
398 [Pérez-Ortega et al., \(2017\)](#) on a list of more than 600 contaminants in various matrices, including  
399 baby foods.

400  
401 This sample preparation protocol could be further transposed to matrices other than baby foods,  
402 only requiring minor adjustments, as baby foods are mixtures of various food commodities of

403 plant and animal origin. Moreover, the AOAC 2007.01 QuEChERS method as well as  
404 MgSO<sub>4</sub>/PSA are widely used for multi-residue screening of organic pollutants in various food  
405 samples (Kim et al., 2019; Musarurwa et al., 2019). Furthermore, the low-starting sample  
406 amount (3 g) coupled to a hydrating strategy enable its further application to matrices with low-  
407 water content ( $\leq 25\%$ ), for which hydration is crucial to ensure effective extraction (Zhang et al.,  
408 2019).

### 410 3.2. *Impact of different detection criteria thresholds on the performances of the* 411 *method*

412 There is no established consensus on the thresholds to be used for  $t_r$  variation and accurate  
413 mass measurement criteria. Moreover, an assessment of these detection criteria is required to  
414 obtain the best compromise between the false positives and false negatives reported by the  
415 software, as explained by (Gómez-Ramos et al., 2013). As a result, different thresholds  
416 detailed in section 2.9. were evaluated. Results regarding the false negative rates are displayed  
417 in Table 1. When  $t_r \pm 0.1$  min and accuracy mass measurement  $\pm 5$  ppm were used, the false  
418 negative rates ranged from 13% to 21% with an average of 16% at 0.01 mg.kg<sup>-1</sup>, and from 3%  
419 to 9% with an average of 5% at 0.1 mg.kg<sup>-1</sup>. When varying only the  $t_r$  threshold to  $\pm 0.5$  min,  
420 the false negative rates ranged from 13% to 20% with an average of 16% at 0.01 mg.kg<sup>-1</sup>, and  
421 from 3% to 9% with an average of 5% at 0.1 mg.kg<sup>-1</sup>. Finally, when setting the  $t_r$  threshold to  
422  $\pm 0.25$  min and accuracy mass measurement to  $\pm 10$  ppm, the false negative rates varied from  
423 9% to 13% with an average of 11% at 0.01 mg.kg<sup>-1</sup>, and from 3% to 4% with an average of  
424 3% at 0.1 mg.kg<sup>-1</sup>. These results demonstrate that a wider  $t_r$  variation with unchanged accurate  
425 mass measurement (5 ppm) did not impact the average detectability, regardless of the  
426 concentration level. However, a larger accurate mass measurement threshold ( $\pm 10$  ppm)  
427 slightly improved pesticide detection, especially at 0.01 mg.kg<sup>-1</sup>. These findings are consistent  
428 with the study of López et al. (2014) which shown the same trends on 199 pesticides in fruits  
429 and vegetables. Otherwise, SDs for each baby food were all  $\leq 7\%$ , suggesting relatively good  
430 inter-batch repeatability. The total inter-batch and inter-matrix SDs were  $\leq 4\%$ , regardless of  
431 the thresholds used, suggesting good comprehensiveness of the method.

432  
433 False positives should also be considered as a criterion for evaluating the effectiveness of a  
434 screening method in HRMS. As there is no requirements in official guidelines (SANTE, 2021;

435 [FDA, 2015](#)) on the false positive rates not to be exceeded, the proposed automated screening  
436 method must be able to provide false positive rates as low as possible, to avoid time-  
437 consuming manual inspections. Results on the false positives are displayed in [ESM \(Table](#)  
438 [S3](#)). When using  $t_r \pm 0.1$  min and accurate mass measurement of  $\pm 5$  ppm, the false positive  
439 rates ranged from 3% to 6% with an average of 5%, and an SD of 2%. When increasing the  $t_r$   
440 threshold to  $\pm 0.5$  min, while keeping the same accurate mass measurement of  $\pm 5$  ppm, the  
441 false positive rates varied from 4% to 8% with an average of 6%, and an SD of 2%. Finally,  
442 when  $t_r \pm 0.25$  min and accurate mass measurement  $\pm 10$  ppm were used, the false positive  
443 rates varied from 9% to 15%, with an average of 13%, and an SD of 5%. Thus, the false  
444 positive rates depended more heavily on the accurate mass measurement used than on the  $t_r$   
445 variation threshold. These results are consistent with the findings of [Pérez-Ortega et al. \(2017\)](#)  
446 and [López et al. \(2014\)](#) regarding the influence of wider accurate mass measurement on false  
447 positives. However, the results were different considering the  $t_r$  criteria, as these authors  
448 showed higher false positives with the widening of the  $t_r$  threshold ([Pérez-Ortega et al., 2017](#);  
449 [López et al., 2014](#)). In conclusion, the thresholds recommended in SANTE guidelines ( $t_r \pm 0.1$   
450 min, accurate mass measurement  $\pm 5$  ppm) ([SANTE, 2021](#)) allowed us to obtain the best  
451 balance between false negative and false positive rates, and were therefore selected for  
452 pesticide detection.

453

454 To the best of our knowledge, this study is the first to provide both false negative and false  
455 positive rate results for multi-residue screening of pesticides in baby food samples. However,  
456 data of this type are available for similar related matrices, such as fruits and vegetables. For  
457 instance, [López et al. \(2014\)](#) proposed a method for the screening of almost 200 pesticides in  
458 a set of 22 fruit and vegetable samples using a QuEChERS procedure followed by UHPLC-Q-  
459 TOF (ESI+) analysis. They were able to detect from 61% to 94% of pesticides with an  
460 average of 84% at  $0.01 \text{ mg.kg}^{-1}$ , i.e., 16% false negatives, when using  $t_r$  of  $\pm 0.2$  min and  
461 accurate mass measurement of  $\pm 5$  ppm. The performances of detection in the present study  
462 and in the one of [López et al. \(2014\)](#) were equivalent (16% false negatives), but considering a  
463 tighter  $t_r$  threshold. On the other hand, they found up to 15 false positives on average against a  
464 library of 504 entries, i.e., a false positive rate of about 3%, which was equivalent to that in  
465 the present work (5%). In the study conducted by [López et al. \(2014\)](#), a QuEChERS  
466 extraction-based protocol was performed using equivalent sample amounts and extraction  
467 solvent volumes, making it possible to obtain 1 g of sample per mL of final extract. In  
468 contrast with the present study, the starting sample mass (3 g) was lower than the



469 subsequently added MeCN (10 mL), providing about 0.3 g of sample per mL of final extract,  
470 which may decrease co-extractive compounds but also increase analyte detection limits. The  
471 overall method provided satisfactory detection performances with low-related variability and  
472 low false positive rate, even at low-level of concentration and given the complex nature of  
473 baby food samples.

474

### 475 3.3. *Impact of a PIL and different MS/MS algorithms on the performances of the* 476 *method*

477 To achieve a simple and reliable automated screening method with unequivocal identification  
478 (level 1 confidence), it is necessary to consider both information regarding precursor ion and  
479 its characteristic fragment ions (Schymanski et al., 2014). For this purpose, the methods  
480 detailed in section 2.5 and the protocols detailed in section 2.10 were evaluated regarding  
481 pesticide identification. The results are displayed in Table 2.

482

483 **Method A** provided false negative rates from 50% to 55% at 0.01 mg.kg<sup>-1</sup>, and from 26% to  
484 28% at 0.1 mg.kg<sup>-1</sup>, depending on the algorithm-score combination used. The false negatives  
485 were higher than those found at detection-stage (Table 1), which is consistent because for  
486 identification, additional parameter, comparison between experimental and library  
487 fragmentation spectra, is taken into account. **Method B** provided false negative rates from  
488 29% to 42% at 0.01 mg.kg<sup>-1</sup>, and from 13% to 17% at 0.1 mg.kg<sup>-1</sup>, depending on the  
489 algorithm-score combination used. **Method B** always provided the lowest false negative rates  
490 at both concentration-levels, which can be explained by the use of a PIL, giving priority to the  
491 pesticides to be fragmented and limiting the loss of MS/MS data. Regarding the fragmentation  
492 triggering rate, it was found that 31% and 19% of the detected pesticides were not fragmented  
493 at 0.01 and 0.1 mg.kg<sup>-1</sup>, respectively with **Method A**. When using **Method B**, the rates of  
494 detected but unfragmented pesticides dropped on average to 8% and 7% at 0.01 mg.kg<sup>-1</sup> and  
495 0.1 mg.kg<sup>-1</sup>, respectively. Interestingly, the PIL did not completely avoid the loss of  
496 information on MS/MS data, preventing these compounds from being unambiguously  
497 identified. However, it considerably overcame this limitation when compared with **Method**  
498 **A**. As a result, **Method B** was retained for data acquisition.

499 Regarding algorithm-score combinations (**Method B**), the false negative rates ranged from  
500 36% at 0.01 mg.kg<sup>-1</sup> to 14% at 0.1 mg.kg<sup>-1</sup> using PUR, from 33% at 0.01 mg.kg<sup>-1</sup> to 14% at  
501 0.1 mg.kg<sup>-1</sup> using FIT, and from 29% at 0.01 mg.kg<sup>-1</sup> to 13% at 0.1 mg.kg<sup>-1</sup> using RFIT, at a

502 similarity score >50%. With a more restrictive similarity score (>70%), the false negative  
503 rates varied from 42% at 0.01 mg.kg<sup>-1</sup> to 17% at 0.1 mg.kg<sup>-1</sup> using PUR, from 37% at 0.01  
504 mg.kg<sup>-1</sup> to 15% at 0.1 mg.kg<sup>-1</sup> using FIT, and from 32% at 0.01 mg.kg<sup>-1</sup> to 14% at 0.1 mg.kg<sup>-1</sup>  
505 using RFIT. This demonstrated that pesticide identification was strongly impacted by the  
506 concentration-level of analytes in the baby food samples. This was expected, since a higher  
507 concentration-level of analyte in the sample leads to better response of precursor and fragment  
508 ions, enhancing pesticide identification. Regarding the different algorithms, the results  
509 showed equivalent performances at 0.1 mg.kg<sup>-1</sup> regardless of the similarity score used. With  
510 lower concentrations, the PUR and FIT algorithms provided equivalent results with a  
511 similarity score >50%, whereas RFIT made it possible to slightly improve the performances.  
512 When using a similarity score >70%, the PUR algorithm showed the worst performances and  
513 RFIT the best. Nevertheless, RFIT is the least restrictive algorithm, because even if expected  
514 fragment ions are not present in the experimental spectrum, they are not taken into account in  
515 the calculation.. On the other hand, PUR is the most restrictive algorithm, as it uses the  
516 fragment ions of both unknown and reference spectra, and their relative abundance for  
517 similarity calculation. This explains why this algorithm provided the highest false negative  
518 rates at low-concentration. The FIT algorithm appeared to be a compromise as it provided  
519 results close to those obtained using RFIT, while providing a higher level of confidence. The  
520 FIT algorithm presents the advantage of considering the entire expected fragmentation  
521 spectrum of the library in the calculation, excluding the interfering fragment ions that can be  
522 found in the experimental spectrum. As an example, the pesticide epoxiconazole ([M+H]<sup>+</sup>,  
523 *m/z* 330.08039, *t<sub>R</sub>* = 6.36 min) was identified with a similarity score of 100% using the FIT  
524 algorithm, but with lower scores (67%) using the PUR and RFIT algorithms due to  
525 unexpected fragment ions (Fig. S4). Thus, the RFIT and PUR algorithms were both discarded  
526 in favour of the FIT algorithm for the identification of pesticides. When using **Method B** and  
527 the FIT algorithm, false negative rates were close either using similarity score >50% or >70%  
528 (Table 2). Thus, a similarity score >70% was retained for the method, as it provides greater  
529 confidence in the automated identification process.

530

531

532 The optimised parameters of identification enabled us to discard all the false positives that  
533 were initially detected with only *t<sub>R</sub>* variation ± 0.1 min and accurate mass measurement ± 5  
534 ppm. However, the fungicide difenoconazole ([M+H]<sup>+</sup>, *m/z* 406.07197, *t<sub>R</sub>* = 7.31 min) initially  
535 considered a false positive, was confirmed to be a true positive at the identification-stage. It

536 was found in non-spiked baby foods 5 and 6. Fig. 3 shows the unexpected identification of  
537 difenoconazole in the non-spiked baby food 5. The mass error was 0.2 ppm, the  $t_r$  variation  
538 was 0 min, the chlorinated-type isotope ratio difference was about 3%, and the similarity  
539 score of identification was about 99% using the FIT algorithm. This demonstrates the  
540 reliability of the developed automated identification method, and how important MS/MS  
541 fragmentation spectra are for unequivocal identification (Schymanski et al., 2014).

542

543 Considering the literature, López et al. (2014) used a QuEChERS-UHPLC-Q-TOF method to  
544 screen almost 200 pesticides in fruits and vegetables. As an identification strategy, the authors  
545 used  $t_r$  variation  $\pm 0.1$  min and accurate mass measurement  $\pm 5$  ppm on the precursor ion, plus  
546 accurate mass measurement  $\pm 10$  ppm on corresponding product ion(s). The average  
547 identification rate was 64% at  $0.01 \text{ mg.kg}^{-1}$ , which was equivalent to our results (37% false  
548 negative rate, i.e., 63% identification rate). Despite the demanding nature of the identification  
549 approach proposed in this study and the complexity of the baby food matrices, the method  
550 showed satisfactory performances for a large-scale screening purpose, even at low-level of  
551 concentration

552

553

#### 554 3.4. Method validation

555 The last part of this work consisted in validation of the complete optimised method, inspired  
556 by SANTE guidelines for screening methods (SANTE, 2021). SDL and LOI, detailed in  
557 section 2.11., were used as criteria of validation. However, as difenoconazole was found in  
558 baby foods 5 and 6, these samples were discarded. The results are displayed in *ESM* (Table  
559 S4) and are summarized in *ESM* (Fig. S5). Regarding the SDL, it was established at  $0.01$   
560  $\text{mg.kg}^{-1}$  for 141 pesticides (71%), and at  $0.1 \text{ mg.kg}^{-1}$  for 48 pesticides (24%). Overall, 95% of  
561 the pesticides had an  $\text{SDL} \leq 0.1 \text{ mg.kg}^{-1}$ . These validation results demonstrated the very good  
562 detection capabilities of the method, and its ability to act as an alert system if a pesticide  
563 found in a baby food sample significantly exceeds the MRL. However, 10 pesticides (5%)  
564 were not detected in at least 29 of the 30 baby food samples. Therefore, their SDL was  
565 established as  $> 0.1 \text{ mg.kg}^{-1}$ .

566

567 Regarding the LOI, it was established at  $0.01 \text{ mg.kg}^{-1}$  for 77 pesticides (39%), and at  
568  $0.1 \text{ mg.kg}^{-1}$  for 68 pesticides (34%). Overall, 73% of the pesticides had an  $\text{LOI} \leq 0.1 \text{ mg.kg}^{-1}$ .

569 However, 54 pesticides (27%) could not be properly identified in at least 29 of the 30 baby  
570 food samples. Therefore, their LOI was established as  $> 0.1 \text{ mg.kg}^{-1}$  (Fig. S5).

571 In the literature, Luz Gómez-Pérez et al. (2015) used  $t_r$  variation  $\pm 0.5 \text{ min}$  and mass error  $< 5$   
572 ppm on the precursor and one of its fragment ions, for the screening of more than 300  
573 pesticides in baby foods. The LOI was established at  $0.01 \text{ mg.kg}^{-1}$  for about 61% of the  
574 compounds. In this study, the authors explained that analytes at low-concentration in complex  
575 matrices may provide fragment ions at low sensitivity, which make the identification process  
576 difficult (Luz Gómez-Pérez et al., 2015). This aspect may have a greater impact on the  
577 performances in the case of our presented study. Indeed, the FIT algorithm used for  
578 identification takes into account all the expected fragment ions of a compound in the  
579 calculation, and not only the most abundant fragment ion(s). At  $0.01 \text{ mg.kg}^{-1}$ , some expected  
580 fragment ions were missing in the experimental fragmentation spectra, which lowers the  
581 similarity scores computed by the software. This could explained the difference in the  
582 performances observed in both studies. In addition, another factor should be highlighted. Luz  
583 Gómez-Pérez et al. (2015) used DIA mode for their screening and identification. In our study,  
584 not all pesticides were fragmented with IDA-PIL mode, preventing them from being  
585 validated. Thus, it would be necessary in a future work to evaluate DIA mode that allows all  
586 precursor ions to be fragmented. Nevertheless, the use of DIA mode would require  
587 optimisation as it provides more interferences and complex spectra that reduce the confidence  
588 into the identification process (Diallo et al., 2022).

589  
590 Regarding validation at the identification stage, the approach proposed in the present study is  
591 not covered by the analytical guidelines, being limited by the options provided in  
592 MasterView<sup>TM</sup> software. In this sense, manufacturers should be able to provide compliant  
593 identification strategies regarding guidelines and the literature. In parallel, there is a need to  
594 improve existing official guidelines (SANTE, 2021; FDA, 2015) to include the identification  
595 approach using MS/MS similarity in addition to the approach already proposed. Furthermore,  
596 there are no requirements regarding false positives for method validation currently. There is  
597 also a need to indicate the false positive rate not to be exceeded to be able to use a screening  
598 method in a high-throughput sample analysis-based context with a high confidence and to  
599 avoid time-consuming manual verifications.

### 600 3.5. Suspect screening analysis

601 HRMS not only offers the possibility of conducting targeted analyses, but also of reprocessing  
602 retrospectively acquired data (Phillips et al., 2018), in order to broaden the analytical scope

603 and optimize monitoring in an exposomic-based context. Thus, the versatility and feasibility  
604 of the method was also demonstrated performing post-acquisition suspect-screening analysis  
605 on the analysed baby food samples. For this purpose, an expanded list of over 500 organic  
606 contaminants including pesticides and metabolites, flame retardants and plasticizers, was  
607 included in the database on the basis of their elemental formula and corresponding exact mass  
608 (Table S5). . Compound identification in suspect-screening analysis was conducted according  
609 to the identification confidence levels proposed by Schymanski et al. (2014). In the first  
610 instance, only the accurate mass measurement  $\pm 5$  ppm was used as a filter (confidence level  
611 5). Among the positive results, if the compound was not fragmented, it was not considered  
612 anymore as a positive result as it was not feasible to identify the contaminant. If the  
613 compound was fragmented, the resulting fragmentation spectrum was compared to the spectra  
614 available in online databases. If the experimental spectrum matched with external spectrum,  
615 the compound was then considered as putatively identified (confidence level 2). Using this  
616 workflow, a flame retardant and plasticizer, tris(2-chloroethyl) phosphate (TCEP), was  
617 putatively identified in some baby food samples. Afterwards, the analytical standard of TCEP  
618 was purchased and injected for confirmation. The criteria used were the  $t_r$  variation  $\pm 0.1$  min  
619 and accurate mass measurement  $\pm 5$  ppm on the precursor ion, as well as the spectral  
620 similarity between the reference and experimental MS/MS fragmentation spectra, using FIT  
621 algorithm with a similarity score  $> 70\%$ . Thus, the presence of TCEP was confirmed at the  
622 confidence level 1 of identification, as shown in Fig.4. The  $t_r$  variation was 0.01 min, the mass  
623 error was -2.5 ppm and the FIT score was 95.7%.

624

625 The proposed method is a qualitative method acting as an alert system, i.e., it is able to detect  
626 and identify the presence or absence of a contaminant. Nevertheless, it would be important to  
627 quantify TCEP, either through a semi-quantitative approach, or precisely through  
628 conventional tandem mass spectrometry to determine whether its presence in the baby food  
629 samples exceeds the MRL.

630

## 631 **4. Conclusion**

632 In this study, a QuEChERS-based protocol coupled to UHPLC-Q-TOF MS (ESI+) analysis  
633 enabled the screening of 204 pesticides in baby foods. The extraction procedure was  
634 optimized based on QuEChERS methods, to set-up a sample preparation protocol that can be  
635 adapted to various baby foods. The QuEChERS based on the AOAC method followed by a

636 purification using MgSO<sub>4</sub>/PSA, provided the less false negatives and the better signal-to-noise  
637 ratio. Screening of pesticides in baby food samples was performed at the detection and  
638 identification stages using a homemade t<sub>r</sub>-MS/MSlibrary, allowing for fast, automated and  
639 unequivocal compound identification. As there is no standardisation, different thresholds on  
640 the t<sub>r</sub> and accurate mass measurement were assessed on the performances of detection.  
641 SANTE guidelines criteria (t<sub>r</sub> ± 0.1 min, accurate mass measurement ± 5 ppm) showed the  
642 best balance between false negatives with 16% and 5% at 0.01 mg.kg<sup>-1</sup> and 0.1 mg.kg<sup>-1</sup>,  
643 respectively, and false positives with a low rate of 5%. Regarding identification, IDA and  
644 IDA-PIL modes as well as different MS/MS algorithms-score combinations were evaluated.  
645 IDA-PIL mode and identification using the FIT algorithm and similarity score >70% were  
646 selected for the method, and demonstrated good performances with 37% and 15% false  
647 negative rates, at 0.01 mg.kg<sup>-1</sup> and 0.1 mg.kg<sup>-1</sup>, respectively. The method was then validated  
648 based on the SANTE guidelines for screening methods: 95% and 73% of the pesticides had a  
649 screening SDL and LOI ≤ 0.1 mg.kg<sup>-1</sup>, respectively. The proposed method enabled the  
650 unequivocal identification of the pesticide difenoconazole and a plasticizer, TCEP, in some of  
651 the investigated samples.

652  
653 Looking forward, the DIA mode should be further evaluated for pesticides and similar  
654 contaminants as it makes it possible to overcome the loss of MS/MS data and the related false  
655 negatives obtained in IDA mode. As the MS/MS spectral similarity is not yet integrated in  
656 analytical guidelines, there is a need to consider this type of identification.

657

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662

## 663 **Declaration of competing interest**

664 The authors declare that they have no conflicts of interest.

665

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## 830 **Figure captions**

831 **Fig.1.** Comparison of different QuEChERS-based procedures for sample preparation  
832 optimisation.

833 **Fig.2.** Impact of different extraction and purification procedures on the false negative rates  
834 (%) obtained for (a) baby food 1; (b) baby food 2; (c) baby food 3; and (d) baby food 4. The  
835 error bars correspond to the standard deviation for the response measured (Mean  $\pm$  SD, n=3).

836 **Fig.3.** Unexpected identification of difenoconazole in non-spiked baby food 5, with  
837 corresponding (a) extracted ion chromatogram; (b) isotopic pattern; and (c) experimental and  
838 reference fragmentation spectra comparison using FIT algorithm.

839 **Fig.4.** Identification of tris(2-chloroethyl) phosphate in non-spiked baby food 1, with  
840 corresponding (a) extracted ion chromatogram; (b) isotopic pattern; (c) experimental and  
841 reference fragmentation spectra comparison using FIT algorithm.

842

# Optimisation of sample preparation

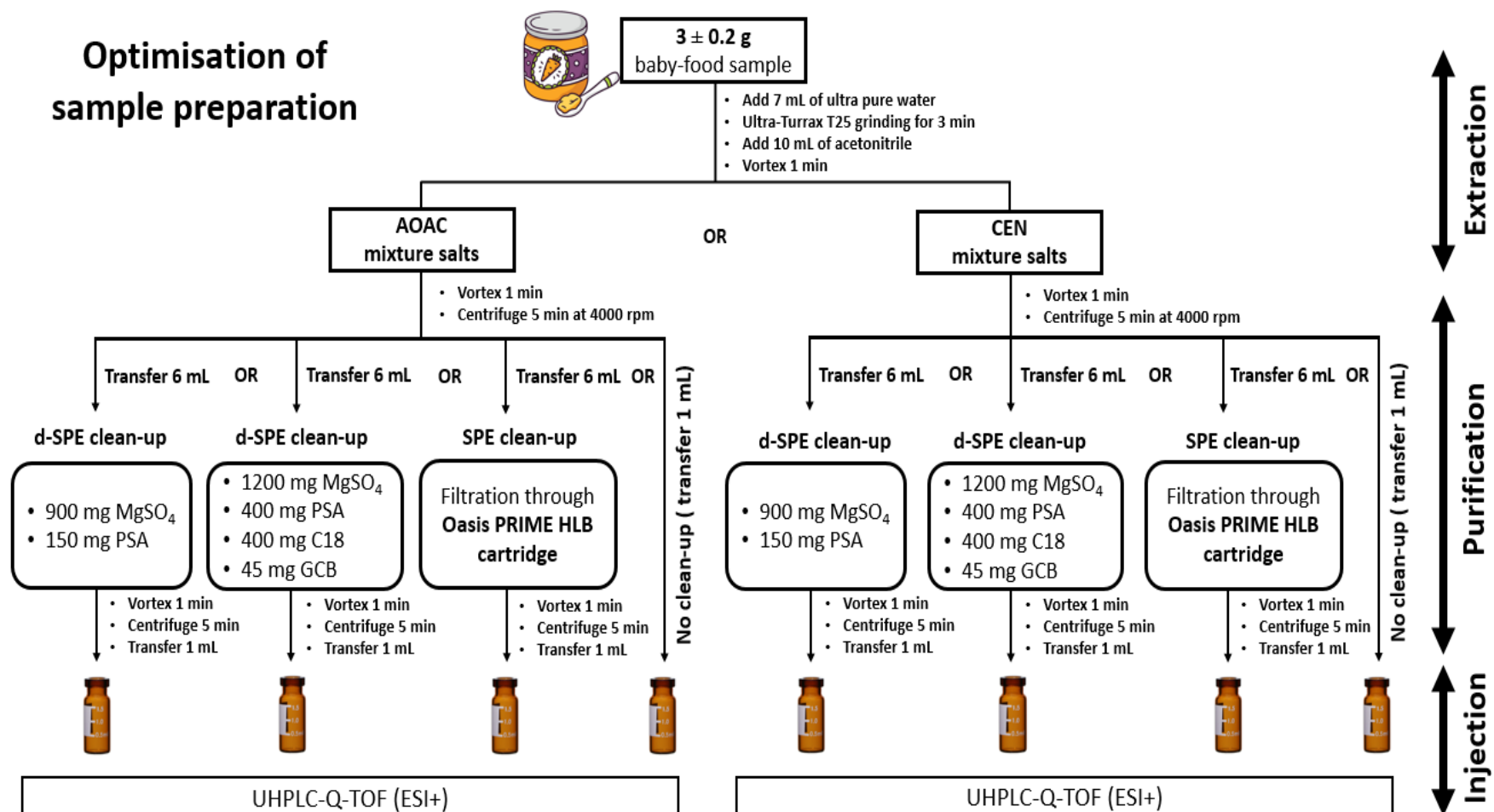
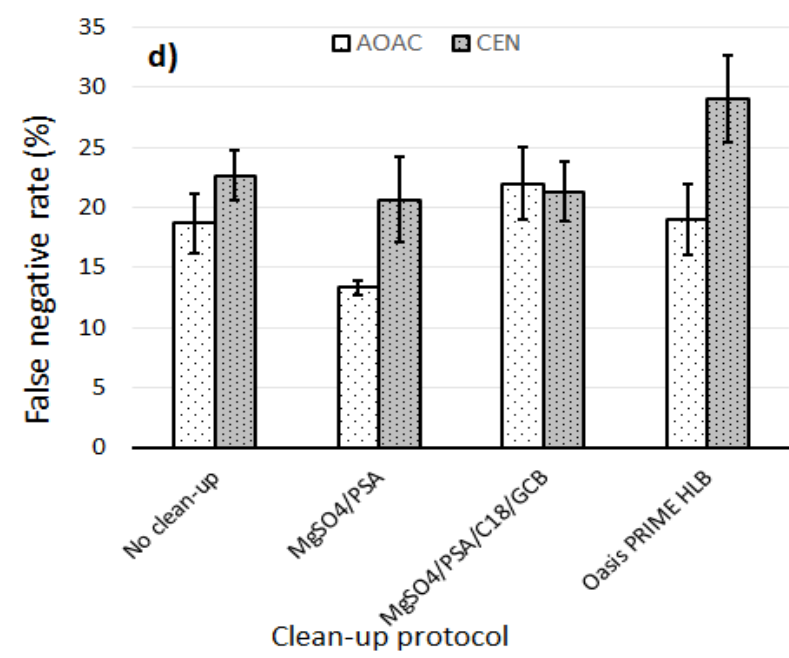
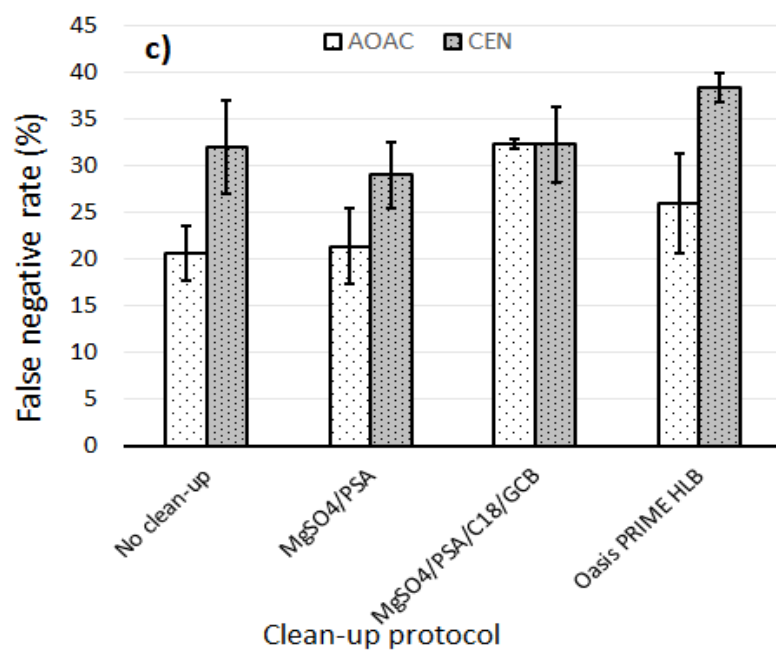
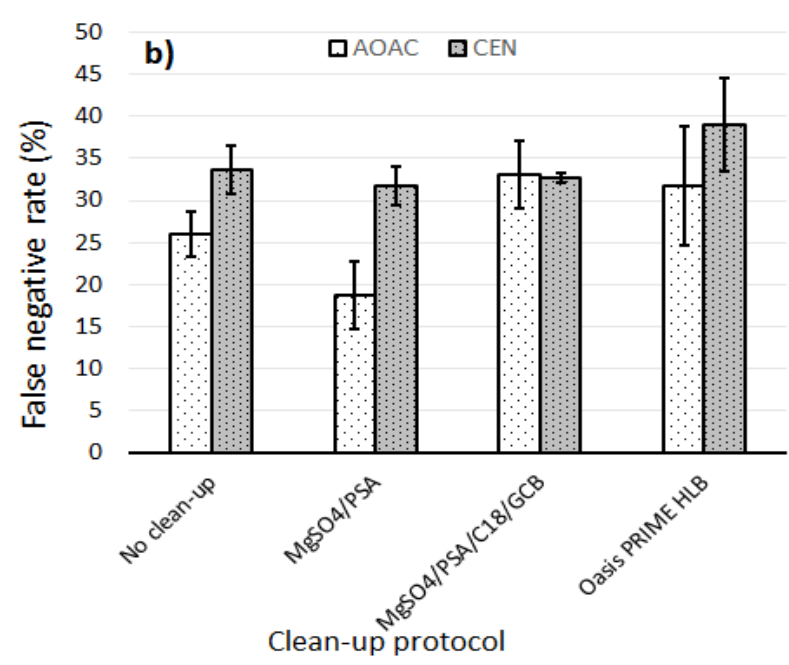
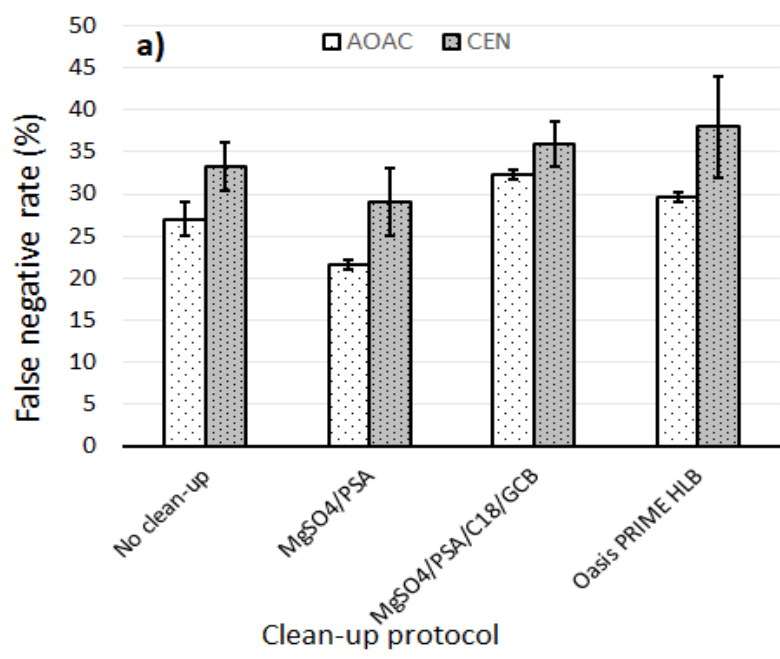
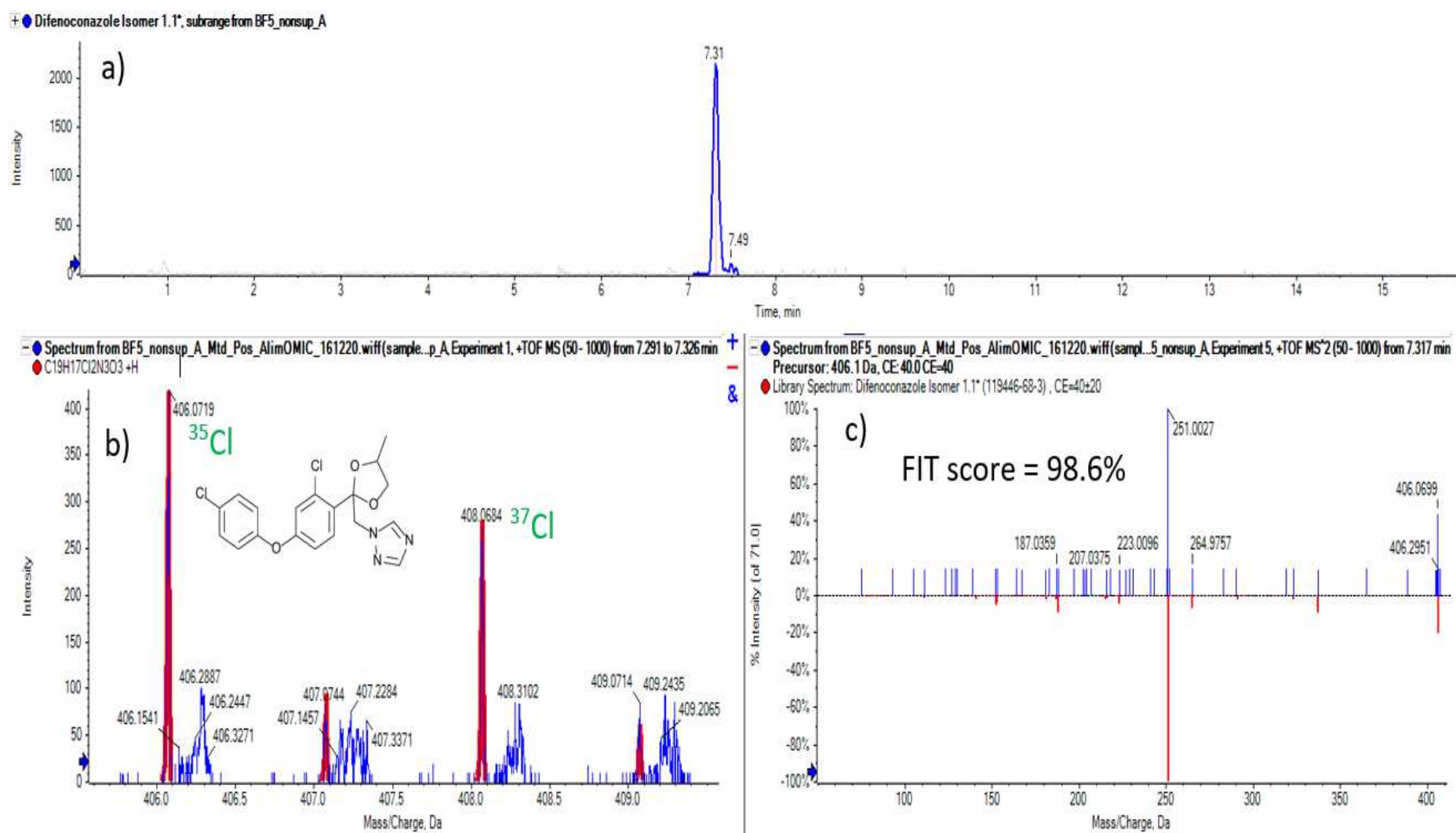


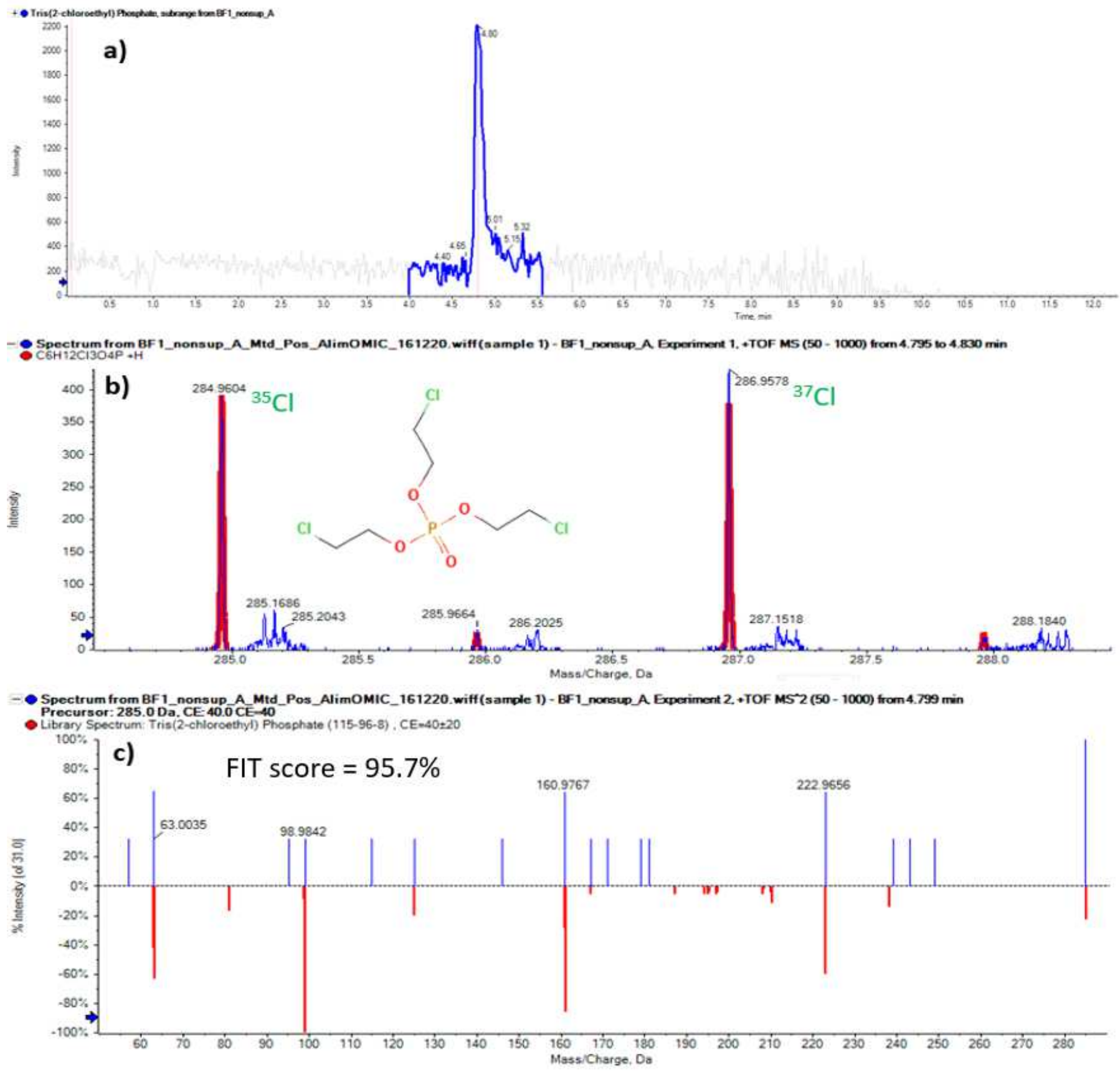
Fig.1.



**Fig.2.**



**Fig.3.**



**Fig.4.**

**Table 1:** Impact of different thresholds on the  $t_r$  and accurate mass measurement criteria regarding the false negatives (%), in a set of 10 baby food samples in triplicate, at 0.01 and 0.1 mg.kg<sup>1</sup>.

False negative (%)* $\pm$ SD (%)						
Reported criteria for detection						
Sample (n=3)	$(t_r \pm 0.1 \text{ min}; \text{ME} \pm 5\text{-ppm})^{[1]}$		$(t_r \pm 0.5 \text{ min}; \text{ME} \pm 5\text{-ppm})^{[2]}$		$(t_r \pm 0.25 \text{ min}; \text{ME} \pm 10\text{-ppm})^{[3]}$	
	Concentration (mg.kg <sup>-1</sup> )					
	0.01	0.1	0.01	0.1	0.01	0.1
Baby food 5	16 $\pm$ 1	6 $\pm$ 2	16 $\pm$ 1	6 $\pm$ 1	12 $\pm$ 2	4 $\pm$ 1
Baby food 6	17 $\pm$ 2	5 $\pm$ 2	17 $\pm$ 2	5 $\pm$ 2	11 $\pm$ 2	4 $\pm$ 2
Baby food 7	15 $\pm$ 4	9 $\pm$ 7	14 $\pm$ 4	9 $\pm$ 7	12 $\pm$ 3	3 $\pm$ 1
Baby food 8	21 $\pm$ 1	5 $\pm$ 1	20 $\pm$ 1	5 $\pm$ 1	12 $\pm$ 3	3 $\pm$ 1
Baby food 9	17 $\pm$ 5	6 $\pm$ 2	16 $\pm$ 5	6 $\pm$ 2	10 $\pm$ 3	4 $\pm$ 2
Baby food 10	16 $\pm$ 7	5 $\pm$ 1	15 $\pm$ 7	5 $\pm$ 1	11 $\pm$ 5	3 $\pm$ 1
Baby food 11	15 $\pm$ 5	5 $\pm$ 2	14 $\pm$ 4	5 $\pm$ 2	9 $\pm$ 5	3 $\pm$ 1
Baby food 12	13 $\pm$ 4	4 $\pm$ 0	13 $\pm$ 1	4 $\pm$ 0	9 $\pm$ 3	3 $\pm$ 0
Baby food 13	17 $\pm$ 6	5 $\pm$ 2	16 $\pm$ 6	5 $\pm$ 2	13 $\pm$ 4	3 $\pm$ 1
Baby food 14	15 $\pm$ 4	3 $\pm$ 0	15 $\pm$ 2	3 $\pm$ 0	10 $\pm$ 4	3 $\pm$ 0
<b>Average (n = 30) <math>\pm</math> SD (%)</b>	<b>16 <math>\pm</math> 4</b>	<b>5 <math>\pm</math> 3</b>	<b>16 <math>\pm</math> 4</b>	<b>5 <math>\pm</math> 3</b>	<b>11 <math>\pm</math> 3</b>	<b>3 <math>\pm</math> 1</b>

<sup>[1]</sup> SANTE/12682/2019; <sup>[2]</sup> FDA, 2015; Wang et al., 2019; <sup>[3]</sup> Pérez-Ortega et al., 2017

\* The percentages were calculated based on 199 compounds as some pesticides had identical formulas and were co-eluted: aldicarb sulfone/butoxycarboxim (C<sub>7</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S); desmedipham/phenmedipham (C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>); prometon/secbumeton/terbumeton (C<sub>10</sub>H<sub>19</sub>N<sub>5</sub>O) and prometryne/terbutryn (C<sub>10</sub>H<sub>19</sub>N<sub>5</sub>S)

**Table 2:** Impact of a PIL and different algorithm-score combinations on the false negatives (%), in a set of 10 baby food samples in triplicate, at 0.01 and 0.1 mg.kg<sup>-1</sup>.

MS/MS Algorithm	Similarity score	False negative (%)* ± SD (%)			
		Concentration (mg.kg <sup>-1</sup> )			
		0.01 (MRL)	Method B	0.1	Method B
PUR	>50%	52 ± 8	36 ± 7	27 ± 4	14 ± 1
PUR	>70%	55 ± 8	42 ± 8	28 ± 4	17 ± 2
FIT	>50%	50 ± 8	33 ± 7	26 ± 4	14 ± 1
FIT	>70%	51 ± 8	37 ± 8	27 ± 4	15 ± 2
RFIT	>50%	50 ± 9	29 ± 6	26 ± 4	13 ± 1
RFIT	>70%	52 ± 8	32 ± 6	26 ± 4	14 ± 1

\* The percentages were calculated based on 199 compounds as some pesticides had identical formulas and were co-eluted: aldicarb sulfone/butoxycarboxim (C<sub>7</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S); desmedipham/phenmedipham (C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>); prometon/secbumeton/terbumeton (C<sub>10</sub>H<sub>19</sub>N<sub>5</sub>O) and prometryne/terbutryn (C<sub>10</sub>H<sub>19</sub>N<sub>5</sub>S)