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► **To cite this version:**

Béla Kmellár, László Abrankó, Peter Fodor, Steven Lehotay. Routine approach to qualitatively screen for 300 pesticides and quantify those frequently detected in fruits and vegetables using liquid chromatography tandem mass spectrometry. *Food Additives and Contaminants*, 2010, 27 (10), pp.1415. 10.1080/19440049.2010.490791 . hal-00603433

HAL Id: hal-00603433

<https://hal.science/hal-00603433>

Submitted on 25 Jun 2011

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Routine approach to qualitatively screen for 300 pesticides and quantify those frequently detected in fruits and vegetables using liquid chromatography tandem mass spectrometry

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2009-414.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	27-Apr-2010
Complete List of Authors:	Kmellár, Béla; Corvinus University of Budapest, Department of Applied Chemistry Abrankó, László; Corvinus University of Budapest, Department of Applied Chemistry Fodor, Peter; Corvinus University of Budapest, Department of Applied Chemistry Lehotay, Steven; USDA ARS, Eastern Regional Research Center
Methods/Techniques:	Chromatography - LC/MS, LC/MS
Additives/Contaminants:	Pesticide residues, Pesticides
Food Types:	Fruit, Mushrooms, Vegetables

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4 1 **Routine approach to qualitatively screen for 300 pesticides and**
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6 2 **quantify those frequently detected in fruit and vegetables using**
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8 3 **liquid chromatography tandem mass spectrometry**
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14 6 Béla Kmellár,^{1*} László Abrankó,¹ Péter Fodor,¹ and Steven J. Lehotay²
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37 19 Disclaimer: Mention of brand or firm name does not constitute an endorsement by the U.S.
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39 20 Department of Agriculture above others of a similar nature not mentioned.
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1
2
3 **Abstract**
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7 This paper describes an efficient and effective analytical scheme to first screen for 300
8 pesticides in fruit and vegetables samples using liquid chromatography tandem mass
9 spectrometry (LC-MS/MS) with a commercial enhanced product ion method. Then presumed
10 positive extracts are analyzed using a quantitative and confirmatory LC-MS/MS method
11 optimized for 55 pesticides. QuEChERS with acetate buffering (AOAC Official Method
12 2007.01) was used for sample preparation, which has been previously shown to yield high
13 quality results for hundreds of pesticide residues in foods. The advantages and disadvantages
14 of both the qualitative screening and quantitative/confirmatory methods and their
15 combination are critically discussed. No false negatives for the 55 pesticides occurred above
16 10 ng g⁻¹ for extracts analyzed by both LC-MS/MS methods, and the no false positives were
17 encountered from the screening analysis (after analyst review) because all presumptive
18 identifications were confirmed in the second analysis. The monitoring scheme was applied
19 during a one-year period on 200 fruit and vegetable samples from Hungarian markets. No
20 pesticide residues were found in half of the samples, and 12 violations of European maximum
21 residue limits were detected.
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41 **Keywords:** pesticides, routine analysis, liquid chromatography, tandem mass spectrometry,
42 instrument software
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45 Introduction

46 Plant protection products (more commonly known as pesticides) are widely used in
47 agriculture to increase the yield, improve quality, and extend storage life of food crops. The
48 pesticides must undergo extensive efficacy, environmental, and toxicological testing to be
49 registered by governments for legal use in specified applications. The applied chemicals
50 and/or their degradation products may remain as residues in the agricultural products, which
51 becomes a concern for human exposure. Therefore, maximum residue levels (MRLs) (or
52 “tolerances” in the U.S.), which limit the types and amounts of residues that can be legally
53 present on foods, are set by regulatory bodies worldwide. In Europe, EU Council Directive
54 91/414/EEC describes the regulatory framework by which MRLs are set. If the farmers apply
55 the pesticides properly on crops for which the pesticides have been registered, and appropriate
56 harvest intervals are given, then it is very unlikely that regulatory limits will be exceeded.
57 Unfortunately, not all farmers follow legal practices, and due to the tremendous number of
58 pesticides and crops in production, there is a need for routine multiresidue pesticide
59 monitoring using methods with wide analytical scope (Fernández-Alba, 2008).

60 The most common techniques in modern multiresidue target pesticide analysis are gas
61 chromatography and liquid chromatography coupled to mass spectrometry (GC-MS; LC-MS)
62 and/or tandem mass spectrometry (GC-MS/MS; LC-MS/MS) with triple quadrupole mass
63 analyzers. The numerous methods available for pesticide analysis shows the importance of
64 this application and rapid pace of developments in analytical chemistry. For example, Agüera
65 described a method nearly 10 years ago for the measurement of only 10 organophosphorus
66 and organochlorine pesticides by GC-MS (Aguera, 2000), but since that time, the number of
67 pesticides typically included in methods has increased dramatically. Reports in the literature
68 on LC-MS/MS described the increasingly wider scope analysis of 19 (Granby, 2004), 32
69 (Pozo, 2007), 52 (Hernandez, 2006), 74 (Ortelli, 2004), 82 (Banerjee, 2007), 160 (Kmellár,
70 2008) or 169 (Pizzutti, 2007) pesticides and/or degradation products in fruit and vegetable
71 samples.

72 The sample preparation techniques have also advanced to complement the analytical
73 techniques depending on the types of analytes and matrices monitored. The “quick, easy,
74 cheap, effective, rugged, and safe” (QuEChERS) method for pesticide residues in foods
75 (Anastassiades, 2003; Lehotay, 2005; Lehotay, 2005; Lehotay, 2007; European Committee for
76 Standardization (CEN) Standard Method EN 15662, 2008) is an example of a method which
77 takes advantage of the powerful features of nearly universal selectivity and high sensitivity of
78 modern GC- and LC-MS(/MS) instruments. The QuEChERS approach has been extensively

1
2
3 79 validated for hundreds of pesticide residues in many types of foods, and has become AOAC
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5 80 Official Method 2007.01 (Lehotay, 2007) and European Committee for Standardization
6
7 81 (CEN) Standard Method EN 15662.

8
9 82 Triple quadrupole MS/MS instruments are mainly applicable for sensitive and
10
11 83 selective quantitative measurements and identification of known, targeted analytes in selected
12
13 84 or multiple reaction monitoring (SRM or MRM) mode. Recently, many new software and
14
15 85 hardware capabilities have been introduced from MS/MS manufacturers to allow faster
16
17 86 analysis times, lower detection limits, more ion transitions per given time, fast
18
19 87 positive/negative switching, and beneficial data handling features. One of the companies,
20
21 88 Applied Biosystems, introduced an enhanced product ion (EPI) feature in MRM mode for
22
23 89 their linear ion trap products using information dependent acquisition (known as data
24
25 90 dependent scanning by other manufacturers) and MS/MS spectral libraries (Applied
26
27 91 Biosystems Application Note 114AP38-02, 2005). Such products should be independently
28
29 92 evaluated in real-world applications to assess their capabilities and limitations.

30
31 93 One of the major problems in LC-MS/MS pesticide analysis due to the typical use of
32
33 94 electrospray ionization (ESI) is caused by matrix effects. Quantitative results in LC-MS/MS
34
35 95 cannot be trusted unless matrix effects have been assessed and compensated for if they are
36
37 96 found to occur (Granby, 2004; Krueve, 2008; Niessen, 2006). The best approach to
38
39 97 compensate for matrix effects entails the use of isotopically-labeled internal standards for
40
41 98 each analyte, but this is not feasible in multiresidue analysis of so many pesticides. Therefore,
42
43 99 the most common approach is to use matrix-matched calibration standards. However, it can
44
45 100 be difficult to find a blank matrix from which to prepare the calibration standards, and
46
47 101 compensation from one sample to another (even for the same matrix) may not be the same. A
48
49 102 method of standard additions in the sample extract may be an alternative approach.

50
51 103 The aim of this study was to assess in real-world practice the combination of two
52
53 104 methods. first, QuEChERS fruit and vegetables extracts were injected in LC-MS/MS using a
54
55 105 qualitative analysis of 300 targeted pesticides in the commercial MRM-triggered EPI
56
57 106 screening method; and secondly for presumptive positives, a second analysis was conducted
58
59 107 using a quantitative/confirmatory MRM method we optimized for 55 previously detected
60
61 108 and/or frequently used pesticides. The method combination and its limitations were critically
62
63 109 discussed and it was tested on 200 fruit and vegetables samples from the Hungarian market.

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66
67 111 **Materials and methods**

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1
2
3 113 *Reagents and materials*

4
5 114 HPLC grade acetonitrile was purchased from Fisher Scientific (Loughborough, UK),
6
7 115 deionized water was obtained from a Milli-Q reagent water system (Billerica, MA; USA),
8
9 116 dimethyl formamide was obtained from Reanal (Budapest, Hungary), anhydrous magnesium
10
11 117 sulfate was obtained from Scharlau Chemie S.A. (Sentmenat, Spain), and PSA-bonded silica
12
13 118 (primary secondary amine) was obtained from Supelco (Bellefonte, PA; USA). Each sample
14
15 119 was filtered through a 0.45 μm PVDF filter (Roth, Carlsruhe, Germany) before injection.
16
17 120 Acetic acid and sodium acetate (both from Merck, Darmstadt, Germany) were used for the
18
19 121 sample preparation procedure. Analytical grade pesticide standards were ordered from
20
21 122 Sigma-Aldrich (Steinheim, Germany), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and
22
23 123 Riedel-de Haen (Germany) and stored at -30°C . Individual standard stock solutions were
24
25 124 prepared by dissolving the crystalline standards in acetonitrile (or dimethyl formamide for
26
27 125 those insoluble in acetonitrile) to reach the final concentration of 1000-4000 $\mu\text{g mL}^{-1}$. For
28
29 126 method optimization, individual standard solutions were used, which were prepared by
30
31 127 diluting the stock solution to a concentration of 1-4 $\mu\text{g mL}^{-1}$. A standard mix solution in
32
33 128 acetonitrile for preparation of calibration standards was prepared from the individual stock
34
35 129 solutions to yield 10 $\mu\text{g mL}^{-1}$. All solutions were kept at -18°C before use.

36
37 131 *Sample preparation*

38
39 132 The acetate-buffered QuEChERS sample preparation method for pesticides (AOAC
40
41 133 Official Method 2007.01) was applied to all the samples (Granby, 2004; Krueve, 2008;
42
43 134 Niessen, 2006). After homogenization with a house-hold mill (equipped with stainless steel
44
45 135 knives), a 15 g portion of the homogenized sample was weighed into a 50 mL PTFE tube and
46
47 136 100 μL of 50 $\mu\text{g mL}^{-1}$ triphenyl phosphate (TPP) surrogate standard solution in acetonitrile
48
49 137 was added followed by 15 mL of acetonitrile containing 1% acetic acid (v/v not accounting
50
51 138 for purity). Then, 6 g MgSO_4 and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of
52
53 139 anhydrous form) were added, and the sample was shaken forcefully for 4 min. The sample
54
55 140 was then centrifuged (Hermle Z206A, Labcompare, San Fransisco, USA) at 4000 rpm (1860
56
57 141 rcf) for 5 min, and 5 mL of the supernatant was transferred to a 15 mL PTFE tube to which
58
59 142 750 mg MgSO_4 and 250 mg PSA were added. The extract was shaken using a vortex mixer
60
143 for 20 s and centrifuged at 4000 rpm again for 5 min. Approximately 3 mL of the supernatant
144 was filtered through a 0.45 μm PTFE filter (13 mm diameter), and 800 μL portions were
145 transferred to autosampler vials. The extracts were evaporated to dryness under a stream of
146 argon and reconstituted in 800 μL acetonitrile/water (20/80, v/v) for the LC-MS/MS analysis.

1
2
3 147 For the matrix-matched and standard addition calibrations, 4 x 80 μL of reconstituted
4
5 148 samples were transferred into autosampler glass inserts, and 20 μL portions of 0; 250; 500 and
6
7 149 1250 ng mL^{-1} standard mix solutions containing the 55 pesticides in 25/75 acetonitrile/water
8
9 150 (v/v) were added to reach the final additional concentrations of 0; 50; 100 and 250 ng g^{-1}
10
11 151 equivalents, respectively.

12 152

13
14 153 *LC-MS analysis*

15 154 For the LC analysis, an Agilent (Little Falls, DE; USA) 1100 HPLC system was used.
16 155 It contained a binary pump, a degasser, column thermostat, and an autosampler. A reverse
17 156 phase C8 analytical column of 150 mm \times 4.6 mm i.d. and 5 μm particle size and a guard
18
19 157 column of 12.5 mm \times 4.6mm and 5 μm particle size (both were Agilent Zorbax Eclipse XDB)
20
21 158 were coupled to the LC system. Deionized water containing 0.1% formic acid (mobile phase
22
23 159 component A) and acetonitrile (component B) were employed for the gradient program,
24
25 160 which started with 20% B for 3 min and was linearly increased to 100% B in 27 min (held for
26
27 161 3 min). The column was then re-equilibrated over 12 min. back to 20% B. Thus, the total run
28
29 162 time took 45 min. The flow rate was constant at 0.6 mL min^{-1} , and injection volume was 10
30
31 163 μL .

32
33 164 For the MS/MS analysis, an Applied Biosystems (Foster City, CA; USA) 3200
34
35 165 QTRAP system was used. It was equipped with a turbo ion spray interface which was
36
37 166 operated in positive mode (ESI+). Its parameters were as follows in the EPI screening
38
39 167 approach for 300 pesticides and MRM quantitation for 55 pesticides, respectively: curtain gas
40
41 168 flow = 20 psi and 10 psi; collision gas = 12 and 5 (arbitrary units); ion spray voltage = 5000
42
43 169 and 5500 V; temperature = 450°C in both cases; ion source gas1 flow = 40 psi and 50 psi, ion
44
45 170 source gas2 flow = 50 psi in both cases; and dwell time of one transition was 5 and 15 ms,
46
47 171 respectively. Nitrogen was applied in the ion source and for the collision gas. In the
48
49 172 screening method, vendor recommended declustering potentials of 20, 35, and 50 V were
50
51 173 applied when the MRM signal automatically triggered the EPI process.

52 174 Applied Biosystems Analyst 1.4.2 software was used for instrument control and data
53 175 processing. For the determination of 300 pesticides, the commercial method of Applied
54 176 Biosystems and its library was used (Applied Biosystems Application Note 114AP38-02,
55
56 177 2005).

57 178

58
59 179 **Results and discussion**

60 180

181 *Concept of qualitative screening and quantitation of pesticide residues*

182 In this study, we describe the combination of two parallel methods: 1) qualitative
183 screening for 300 target pesticides by LC-MS/MS using MRM data dependent triggered EPI
184 mode with the commercial spectra database; and 2) confirmation and quantitative
185 determination of the frequently used and/or previously detected pesticides using the MRM
186 method we developed for 55 pesticides. Compared to possible alternatives available to us
187 within our budget constraints, this concept was believed to give widest scope with the least
188 effort, and still give excellent qualitative and quantitative results, particularly when using
189 QuEChERS for sample preparation. We sought to evaluate and implement the approach using
190 commercial samples from the market. For efficiency and convenience, the two methods use
191 the same instrument working with the same column and using the same gradient program.

192 The workflow of the concept is shown in Figure 1, and the process is as follows: 1)
193 conduct AOAC Official Method 2007.01 and analyze the extract using the EPI screening
194 method for 300 pesticides; 2) if no pesticides are detected in the sample, no other
195 measurements are necessary; 3) if any of the 55 pesticides from the developed MRM method
196 are found to be positive in the EPI analysis, then re-inject the extract using the MRM method;
197 4) those presumptive positive residues are confirmed and quantified (or found not to be
198 present) using reference standards and a method of standard additions; 5) when a target
199 pesticide is found to be positive in the EPI method but is not included among the 55 MRM
200 analytes, then a reference standard of the pesticide is obtained, optimal ESI+ conditions are
201 determined, and the MRM method is modified to include it for further qualitative and
202 quantitative analysis. In this way, the MRM list of pesticide analytes grows over time.

203 Initial false positive results from the screening method can be uncovered with this
204 approach by comparing retention times between the methods, and findings that don't match
205 are rejected. Furthermore, the peak shapes must be the same, the MS/MS product ion
206 transitions must have similar ratios as the reference standards, the signal/noise (S/N) ratios for
207 all ion peaks must be >10, and quantitation must give a concentration greater than the
208 reporting limit. Quantitation, identification, and confirmation of chemicals using MS
209 techniques are discussed in the literature (Lehotay, 2008; Bethem, 2003; Milman, 2005;
210 Document No. SANCO/2007/3131).

211 A major drawback in the use of matrix-matched calibration involves the need to find
212 blanks for the matrices needed, thus we chose to apply a method of standard additions using
213 multiple aliquots of the final sample extracts. This entailed taking three additional 80 μ L
214 aliquots of presumptive positive extracts and adding standards to achieve final additional

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2
3 215 equivalent concentrations 50, 100 and 250 ng g⁻¹, which were measured by the MRM method.
4
5 216 We checked that the slopes of all detected pesticides were linear, which was nearly always the
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7 217 case for original sample concentrations less than about 200 ng g⁻¹, but if the slope was not
8
9 218 linear for higher pesticide concentrations in the sample, we diluted the extracts 5- or 10-fold
10
11 219 to reach the linear region. After one year of routine application of this technique, we
12
13 220 generated a calibration-slope database for the 55 investigated pesticides in 12 different fruit
14
15 221 and vegetable matrices.
16
17 222

223 *Screening identification of 300 pesticides in EPI*

18
19 224 In the commercial EPI approach we followed, a chosen (usually the most intensive)
20
21 225 transition of each targeted analyte is monitored, which translates to 300 transitions in this
22
23 226 method. If the intensity of the ion transition signal exceeds the chosen intensity threshold of
24
25 227 500 counts per second (cps), then the quasi-molecular ion is fragmented at 20, 35, and 50 V
26
27 228 collision energies to yield product ions, which are further evaluated for possible positive
28
29 229 findings (this technique is termed information dependent analysis by Applied Biosystems and
30
31 230 data dependent scanning or other terms by other vendors). The detection limits depended on
32
33 231 the 500 cps threshold in our study, and naturally, the system would be less sensitive if a
34
35 232 higher threshold was used.

36
37 233 The total ion current (TIC) chromatogram consists of the intensities of all 300
38
39 234 monitored transitions plus any additional product ion signals that arise if the threshold was
40
41 235 exceeded. Searches and presumptive identifications of pesticides from the TIC were started
42
43 236 manually in this version of software (Analyst 1.4.2.). An advantage of the EPI approach is
44
45 237 that MS/MS spectral library searching and comparisons are done automatically by the
46
47 238 software for the 20, 35, and 50 V collision energy spectra, but it allows checking and review
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49 239 by the analyst. Several criteria have to be met simultaneously for the presumptive
50
51 240 identifications (also known as indications (Lehotay, 2008)) to be made: 1) a peak from the
52
53 241 precursor and product ion transitions must all have the same retention time; 2) the
54
55 242 characteristic fragmentation pattern of ions and their ratios obtained by the 3 different
56
57 243 collision energies have to match a compound in the library; and 3) the actual molecular
58
59 244 weight of the pesticide presumptively identified has to correlate with the quasi-molecular ion
60
245 of the precursor ion in EPI. This means that if the molecular weight of the pesticide in the
246 library is [M], then the quasi-molecular ion in ESI+ must originate from [M+H]⁺ (M+1) or
247 [M+NH₄]⁺ (M+18) in a few cases, such as carbamates in the spectral library. In practice, the
248 hydrogen adduct [M+H]⁺ occurs almost exclusively in this application.

1
2
3 249 Figure 2 shows an example for an EPI library-based identification of the pesticide
4 azoxystrobin from a cabbage sample extract. This example shows the evaluation of a peak
5 250 obtained at the retention time of 22.0 min. As observable in boxes (i) and (ii) in Figure 2, the
6 251 software detected an enhanced signal in the TIC that exceeded the intensity threshold of 500
7 252 cps, which triggered collision activated dissociation of the most intense precursor ion at the
8 253 given 3 collision energy voltages (default values of 20, 35, 50 V were used). As shown in
9 254 section (iii) of Figure 2, each averaged spectrum represent characteristic fragmentation
10 255 patterns from the precursor ion. The m/z 404.5 ion that appears in the 20 V spectrum very
11 256 likely indicates the quasi-molecular ion of the chemical of interest. The other two EPI spectra
12 257 at increasing collision energies show how the m/z 404.5 ion disappears while ions of lower
13 258 m/z (fragments of the probable quasi-molecular ion) increase. Box (iv) shows the extracted
14 259 ion chromatogram for the m/z 404 to m/z 372 transition, which is the most intense and
15 260 probably indicates the loss of a methoxy group from the molecule.
16 261

17 262 In this example in Figure 2, the MS/MS spectral library search of just the 35 V
18 263 averaged spectrum is shown (see box v). The software calculates the matching factors for the
19 264 possible hits in the library and lists them on the screen. The findings can be sorted by name,
20 265 formula, molecular weight, reverse fit, fit, purity, and collision energy. The collision energy
21 266 is given along with the library spectra because different spectra are obtained at the different
22 267 conditions. The software-calculated fit, reverse fit, and purity values are given in terms of
23 268 percentage (fit and reverse fit relate to the spectral match of the sample spectrum to the library
24 269 spectrum, and *vice versa*, respectively, and purity relates to the presence of extra ions in the
25 270 sample spectrum that are not included in the library spectrum).

26 271 We analyzed many different types of fruit and vegetable matrices in experiments, and
27 272 we found that the software worked well on its own to provide the top hit for pesticides in the
28 273 library. The analyst needed only to review the retention times for the selected ion peaks for
29 274 the indicated pesticide. In the example shown in Figure 2, azoxystrobin and its most intense
30 275 first product ion transition, m/z 404 to m/z 372, is presented. The peak for that transition as
31 276 shown in box (iv) gave a prominent Gaussian peak, and the analyst decided to re-analyze the
32 277 extract in the MRM method, which included azoxystrobin.
33 278

34 279 ***False indications***

35 280 False presumptive positives are when the pesticide is indicated to be present by the
36 281 screening method (including analyst review), but is not detected by the MRM method. Many
37 282 instances occurred when a positive precursor ion in the screening method would trigger the

1
2
3 283 EPI function to occur, but the automated library search and analyst review of the spectra
4
5 284 eliminated false presumptive positives. The EPI was “falsely triggered” (threshold exceeded,
6
7 285 but no pesticide was identified) most commonly when the TIC contained extensive chemical
8
9 286 noise from the matrix background. These matrix components sometimes gave similar
10
11 287 fragmentation patterns as pesticides in the library, such as a false indication of the mass
12
13 288 spectrum for fenitrothion in lettuce extracts, but they were recognized and eliminated by the
14
15 289 analyst with practice during manual evaluation. We deemed it better to re-inject the sample
16
17 290 using the MRM method whenever we had reasonable doubt if a pesticide was present or not,
18
19 291 but in practice, there was very little reasonable doubt, and none of the re-injected samples
20
21 292 from the EPI screening method gave false positives in the MRM confirmation and
22
23 293 quantitation method.

24
25 294 Possible interferences from matrix are known to increase with respect to lower masses
26
27 295 (*e.g.*, $m/z < 200$), and smaller pesticides tend to yield only a single product ion transition.
28
29 296 Compounds with low mass or that yield only one fragment ion had greater potential for false
30
31 297 positives (and false negatives) in general. An example of this is diphenylamine with MW
32
33 298 =169.2 amu, which gives only m/z 170 to m/z /93.0 transition. In this case, the quasi-
34
35 299 molecular ion disappears even using the lowest 20 V collision energy, which makes it difficult
36
37 300 to detect in the EPI method. In EPI, if a pesticide is hidden behind a peak in the TIC, only
38
39 301 one characteristic transition will be seen, but in the MRM method, softer collision energy can
40
41 302 be used to obtain two transitions..
42
43 303

40 304 ***False negatives***

42 305 Sometimes, when we analyzed for the pesticides in the MRM method for the 55
43
44 306 pesticides, identifications would be made for pesticides that were not indicated in the EPI
45
46 307 method for 300 pesticides. False negatives mostly depended on the sensitivity difference
47
48 308 between the two applied methods (see the LOD values in Table 1). The MRM method only
49
50 309 found pesticides missed by the EPI screening method at low concentration levels (*e.g.*, 10 ng
51
52 310 g^{-1}). For example boscalid in carrot (33 ng g^{-1}), in orange (17 ng g^{-1}) and in grapes (29 ng g^{-1})
53
54 311 $^{-1}$), quinoxifen in grapes (10 and 12 ng ng^{-1}) and triadimenol in grapes (18 ng ng^{-1}) were
55
56 312 detected solely by the MRM method. We do not know how many times false negatives
57
58 313 occurred when the extracts were not re-analyzed by the MRM method, but the lack of glaring
59
60 314 false negatives for pesticides at high concentrations was a good sign that the EPI approach
61
62 315 performed well.

1
2
3 316 Otherwise, the possibility of false negatives increases if the threshold value (500 cps in
4
5 317 this method) were enhanced, because it results in higher LODs in the EPI approach. We
6
7 318 found that this function to be critical; and 500 cps was optimal to obtain sufficiently low
8
9 319 detection limits. A lower setting caused an increase in the number of peaks appearing in the
10
11 320 TIC that created more manual work for the analyst. Naturally, new generation instruments
12
13 321 with similar functions are more sensitive (and faster).

14 322 A likely reason for the lack of apparent false positives was the manual evaluation of
15
16 323 the TIC in the screening method, which required a high level of attention by the analyst to
17
18 324 check for peaks for known ion transitions at known retention times for known pesticides.
19
20 325 High background levels in some cases made this very difficult, especially for commodities
21
22 326 with high fat (*e.g.*, avocado) or high flavor and volatile (*e.g.*, onion, garlic and ginger)
23
24 327 composition.

25 328

26 329 *Achievements and pitfalls*

27
28 330 As in every method, this screening approach has advantages and disadvantages. One
29
30 331 advantage is that pesticide reference standards are not required for all 300 targeted analytes in
31
32 332 the library. Presumptive identifications are still possible for pesticides in the extracts (and
33
34 333 potentially other chemicals that could be included in the MS/MS library). Extending the
35
36 334 method to new analyte(s) is possible as new standards are obtained. Another advantage is that
37
38 335 the method can screen for 300 pesticides, which is more than typical in monitoring methods,
39
40 336 and extensive validation is not needed for pesticides that are not detected (albeit it is
41
42 337 important to show that a pesticide analyte will be detected if it is present). In many cases, no
43
44 338 pesticides are found in the fruit and vegetable samples, or only one or a few pesticides will be
45
46 339 present. If the extract has few matrix components, then the manual evaluation of the given
47
48 340 TIC can be conducted easily.

49
50 341 The main disadvantage with any targeted triple quadrupole method is that the list of
51
52 342 analytes in the library is limited, and true unknown analysis cannot be conducted. The
53
54 343 approach is blind to non-targets, unlike in the case of TOF techniques. Another disadvantage
55
56 344 is that the screening method is time-consuming and can be problematic when the matrix is
57
58 345 very complicated.

59
60 346 Another pitfall in the EPI screening method occurs when two target compounds co-
347
348 elute. Only the most intense precursor ion will trigger the EPI process for that *m/z*, and the
349
lower intensity ion, which may also exceed the 500 cps threshold, will not be detected. This
can also occur if an intense matrix peak at a different *m/z* masks the precursor ion of a

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3 350 pesticide analyte. The best example of analyte-analyte co-elution is TPP ($t_R = 25.8$ min) and
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5 351 pyraclostrobin ($t_R = 26.0$ min), which is presented for apple in Figure 3. Pyraclostrobin is
6
7 352 hardly visible in the TIC because it is masked behind the the large TPP peak. A small tailing
8
9 353 effect can be seen in the enlarged picture of the TPP peak. The 3 EPI spectra at 25.8 min
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11 354 show a clear fragmentation pattern, which is known to correspond to the TPP added in all
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13 355 samples at 0.333 mg kg^{-1} during extraction. Of course, the identification of TPP is expected
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15 356 (required for quality control purposes) in all samples, but the software cannot find
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17 357 pyraclostrobin at low concentrations appearing 0.15 min later than the TPP peak apex, despite
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19 358 the different fragmentation ions (327.1/152.2 for TPP vs. 388.2/194.3 for pyraclostrobin).
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21 359 The EPI function only fragments the quasi molecular ion of the more intensive transition,
22
23 360 thus, the transition for pyraclostrobin's fragmentation pattern was not recorded in this case.
24
25 361 The analyst noticed that pyraclostrobin also appeared in the apple sample extract, which was
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27 362 confirmed by the MRM method and determined to have a concentration of 0.069 mg kg^{-1} .
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29 363 Due to this "pitfall," the analyst must take extra care to review the EPI screening
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31 364 chromatograms for the presence of pyraclostrobin near the TPP peak in all samples.
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33 365

366 ***Confirmation of frequently detected pesticides in MRM mode***

367 *Confirmation*

368 For confirmation and quantitation purposes, the two most intensive transitions for the
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370 55 more commonly detected pesticide analytes in the MRM method are monitored. The
371
372 optimized conditions were entered into the method file as given in Table 1 (only one
373
374 transition could be obtained in the case of haloxyfop), which totaled 111 transitions. Table 1
375
376 also lists retention times, peak widths, ion ratios, and limits of detection (LODs) for the
377
378 analytes. The quasi-molecular ions were $[M+H]^+$ (M+1) in all cases except for propargite,
379
380 which had a strong ammonium adduct $[M+NH_4]^+$ (M+18).

375 *Achievements and pitfalls*

376 The MRM confirmation/quantitation method targeted those residues known to have
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378 occurred in Hungarian and secondarily in European markets (Data Pool of the Community
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380 Reference Laboratories for Residues of Pesticides), and consequently these are targeted from
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382 the aspect of their frequent occurrences. It was not worth the effort to include all 300 analytes
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384 in the MRM method and QuEChERS has already been extensively validated in many labs.
385
386 Thus, we chose the 55 most prominent pesticides, which were those that had been previously
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388 detected in the monitoring program. The selectivity and sensitivity of the MRM method are
389
390 better than the EPI method, as the LODs in Table 1 demonstrate (LODs of the 245 other

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3 384 compounds in EPI were not measured, but they are believed to be within the range of the 55
4
5 385 shown). The LODs were estimated by injecting 1; 4; 10; 20; and 40 ng g⁻¹ equivalent
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7 386 standard mix solutions (not sample extracts), and when both transitions for an analyte in the
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9 387 MRM method gave an observable signal peak at the correct t_R, then that concentration was
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11 388 assigned to be the LOD. In the EPI screening method, the LOD estimation required
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13 389 preparation of 5 mixture solutions 11 pesticides each, which were chosen to avoid co-elutions.
14
15 390 The lowest concentration at the pesticide could be identified by the screening method was
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17 391 recorded as the LOD.

18 392 The reported LODs are well below the MRLs for those pesticide that are registered in
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20 393 typical fruits and vegetables, and the 0.01 mg kg⁻¹ level is often reached for those pesticides
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22 394 that are not registered in the commodities analyzed. It is not likely that false negatives above
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24 395 the reporting limits create a problem in our monitoring approach for LC-amenable pesticides,
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26 396 particularly for those pesticides with MRLs.

27 397 Matrix interferences are difficult to predict, but the analyst can be made aware of
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29 398 possible analyte-analyte interfering transitions at similar t_R. For example, the potential
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31 399 interfering transition of azinphos-methyl and phosmet is presented in Figure 4. Whereas, the
32
33 400 317.9/160.2 and the 318.0/160.0 transitions can interfere with each other, their identification
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35 401 in the MRM method relies also on their slightly different t_R and 2nd ion transitions. To meet
36
37 402 identification criteria, the ion ratios for the pesticide transitions must fall within the intervals
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39 403 listed in the laboratory quality control guidelines in the European SANCO directive
40
41 404 (SANCO/2007/3131, 2007).

405

42 406 *Matrix effects and calibration slope database*

43
44 407 The “Achilles heel” of LC-MS/MS for quantitative purposes are matrix effects,
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46 408 typically from ion suppression in ESI mode. Matrix effects lead to uncertainty in the accuracy
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48 409 of the quantitative results, and matrix-matched calibration is the most common approach used
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50 410 to account for matrix effects. The need for blanks and extra extractions required for matrix-
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52 411 matching is not convenient and there are no guarantees that the matrix compensation is the
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54 412 same between the different samples. When multiple matrix types appear in the same
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56 413 sequence, it is questionable if one matrix can be used to represent another (the practice is
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58 414 questionable even within the same matrix type). However, matrix-matching is felt to be more
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60 415 practical compared to the few alternatives in routine multiclass, multiresidue analysis, and
416 matrix-matching has become an established custom (Kmellár, 2008). Indeed, results from

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3 417 proficiency testing in pesticide analysis generally demonstrates that matrix-matching gives
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5 418 acceptable accuracy.

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7 419 The method of standard addition calibration is another alternative, but this also has can
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9 420 be questioned due to potential nonlinearities in the calibration range of the analysis. We
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11 421 chose to spare ourselves the time and materials needed for additional sample preparation of
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13 422 blanks for matrix-matching by taking additional aliquots of each presumptive positive extract
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15 423 for the method of standard additions. All 55 pesticides were added to each extract for
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17 424 confirmation and quantitation in the MRM method even when only a single pesticide was
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19 425 indicated in the EPI screening method. In this way, we generated a large amount of data for
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21 426 calibration experiments and comparisons.

22
23 427 At first, we constructed a database about the obtained calibration slopes originating
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25 428 from 12 selected matrices. A total of 10 calibrations were averaged per each pesticide in each
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27 429 matrix, which were performed in the analyses of the 200 samples over the course of a year.
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29 430 The comparison of slopes over time showed a high variation for many pesticides even for the
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31 431 same matrix when the signals were not normalized to the surrogate standard, TPP. To track
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33 432 method performance, we constructed a control chart of peak areas of TPP, which was added at
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35 433 0.333 mg kg⁻¹ to all samples. As shown in Figure 5, a recognizable change occurred after 90
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37 434 samples when the source was thoroughly cleaned and detector voltage changed from 2400 to
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39 435 2500 V (maximum = 3200 V). The curtain plate of the ion source was usually cleaned once
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41 436 per week, but at this time, we also cleaned the orifice plate and the Q0. Considering that the
42
43 437 fluctuations of TPP intensities included sample preparation as well as instrument performance
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45 438 aspects, the TPP signal fluctuations were reasonably small. Thus, both the sample preparation
46
47 439 and the MRM method were performing quite well, especially considering that so many
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49 440 samples and sample types were analyzed.

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51 441 By normalizing the peak areas of the pesticides to peak areas of TPP for each
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53 442 calibration point, the consistency of day-to-day calibration slopes improved. Table 2 presents
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55 443 the TPP-normalized matrix-matched calibration slopes for the 12 different matrices tested.
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57 444 The 54 pesticides (oxamyl was too insensitive for inclusion) are listed in order of t_R to
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59 445 possibly infer large matrix co-eluting peaks that induced ion suppression in that part of the
60
446 chromatogram (of course, TPP could also be affected by matrix co-elutants, which
447 complicates interpretation of the results). The values in bold designate average slopes when
448 RSD >20%. Many of the pesticides gave very consistent slopes from day-to-day and matrix-
449 to-matrix, as observable by the overall average slope \pm standard deviation column in Table 2
450 among the 12 matrices. Those pesticides with consistent slopes likely have little or no matrix

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3 451 effects in the different matrices. Conversely, some pesticides gave highly variable slopes in
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5 452 all matrices, which indicated matrix effects or, more likely and simply, that the pesticide is
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7 453 less stable or has more problematic analysis. This is expected in routine multiclass,
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9 454 multiresidue analysis, especially over the course of a year at different instrument maintenance
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11 455 conditions. Even for those pesticides with variable slopes within a particular matrix, only a
12
13 456 few pesticides gave overall average slopes with >20% RSD (shown in bold text) among the
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15 457 12 matrices tested.

16 458

17 459 ***Results of a one-year routine application***

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19 460 During a one-year routine application of this monitoring approach (March 2008 to February
20
21 461 2009), 200 samples from the Hungarian market were analyzed (15-20 samples/month). In all
22
23 462 13 types of fruit, 14 types of vegetables, and 10 other sample types were analyzed, as listed in
24
25 463 Table 3. The confirmed positive results sorted by pesticides and their concentration range in
26
27 464 the samples are presented in Table 4. Vegetable samples originated mainly from Hungary and
28
29 465 nearby EU countries, but a large number of fruit samples, especially tropical fruits, were
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31 466 imported from southern European countries (*e.g.* Spain, Italy, and Greece) or non-EU
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33 467 countries in Central or South America, Africa, and Turkey. Exactly half of the samples gave
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35 468 positive confirmations for at least 1 residue (up to 5 pesticides were found in one sample), and
36
37 469 none of the 300 LC-amenable pesticides were detected in the other half.

38
39 470 The most frequently detected pesticides were the fungicides imazalil and
40
41 471 thiabendazole in fruits and azoxystrobin in vegetables. Diphenylamine was detected in the
42
43 472 samples independently from the commodity (we checked the tubes, acetonitrile, water and
44
45 473 other reagents for contamination of diphenylamine, but found that everything was clear).
46
47 474 Among these, the determined concentrations of azoxystrobin were relatively low (0.01-0.55
48
49 475 mg kg⁻¹), while thiabendazole and imazalil often were found at levels as high as 0.90 and 6.66
50
51 476 mg kg⁻¹, respectively. However, none of these results exceeded the current EU MRLs.
52
53 477 Prochloraz was found at high concentration in 3 lemon samples (from Argentina and Turkey)
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55 478 and nicobifen was found to occur at the MRL (10 µg kg⁻¹) in a cabbage sample from
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57 479 Hungary.

58
59 480 Diphenylamine mostly appeared in samples during the summer months at low
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481 concentrations (0.006-0.202 mg kg⁻¹), and the appearance was independent from the country
482 of origin and the commodity. Besides being a fungicide, diphenylamine is widely used as a
483 rubber antioxidant and accelerator, solid fuel rocket propellant, stabilizer for explosives, for
484 preparation of pharmaceutical and veterinary medicine, as a storage preservation of apples,

1
2
3 485 and as a reagent in analytical chemistry. It is conceivable that the stored fruits and vegetables
4
5 486 were treated with diphenylamine to preserve their quality and freshness, or that it was a
6
7 487 contaminant that did not originate from a pesticide application.
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9 488

10 489 **Conclusions**

11 490 The developed combination of the two described methods permitted the fast and easy
12 491 qualitative screening of 300 target pesticides in a 45-min LC-MS/MS run. Albeit the manual
13 492 evaluation of the given chromatograms increased the analysis time by an additional 10 min
14 493 per sample, very little time, costs, and labor was spent on sample preparation. In the case of
15 494 dirty samples, some false indications were observed, but these were caught by the use of the
16 495 MRM confirmatory and quantitative method for the 55 more common pesticides. The
17 496 construction of standard addition calibration standards was carried out with the same extract
18 497 that was injected for screening the 300 compounds previously. A large calibration database in
19 498 different matrices were collected to show the consistency of the average calibration slope,
20 499 which helped us check the accuracy of the calculated results from the method of standard
21 500 additions.
22

23 501 The one-year routine application of this scheme was a comprehensive study that
24 502 showed consumer exposure of pesticides from fruits and vegetables that can be purchased in
25 503 the Hungarian market. Carbendazim, dimethomorph, diphenylamine, nicobifen, haloxyfop,
26 504 diazinon and dimethoate exceeded the current EU MRLs a total of 12 times among the 200
27 505 samples analyzed.
28

29 506 30 507 31 508 **5. Acknowledgments**

32 509
33 510 The authors thank André Schreiber for helpful information about the MRM-triggered
34 511 EPI approach.
35

36 512 37 513 38 514 **6. References**

39 515
40 516 Agüera A, Piedra L, Hernando M D, Fernández-Alba A R, Contreras M. 2000. Splitless large-
41 517 volume GC-MS injection for the analysis of organophosphorus and organochlorine pesticides
42 518 in vegetables using a miniaturised ethyl acetate extraction. *Analyst* 125 1397–1402.

- 1
2
3 519 Anastassiades M, Lehotay S J, Štajnbaher D, Schenck F J. 2003. Fast and Easy Multiresidue
4 Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase
5 520
6 521 Extraction” for the Determination of Pesticide Residues in Produce Journal of AOAC Int.
7
8 522 86(2) 421-431.
9
10 523 Applied Biosystems Application note 114AP38-02. 2005. Available from:
11 524 http://www.absciex.com/LITERATURE/cms_041987.pdf
12
13 525 Banerjee K, Oulkar D P, Dasgupta S, Patil S B, Patil S H, Savant R, Adsule P G. 2007.
14 526 Validation and uncertainty analysis of a multi-residue method for pesticides in grapes using
15 527 ethyl acetate extraction and liquid chromatography–tandem mass spectrometry. Journal of
16 528 Chrom. A 1173 98–109.
17
18 529 Bethem R, Boison J, Gale J, Heller D, Lehotay S J, Loo J, Musser S, Price P, Stein S. 2003.
19 530 Establishing the Fitness for Purpose of Mass Spectrometric Methods. J. of Am. Soc. Mass.
20 531 Spectrom. 14 528–541.
21
22 532 Data Pool of the Community Reference Laboratories for Residues of Pesticides. Available
23 533 from: <http://www.crl-pesticides.eu>
24
25 534 Directive 91/414/EEC Concerning the placing of plant protection products on the market.
26 535 Official Journal of European Community No. 230/1. Available from: [http://eur-](http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1991L0414:20070201:EN:PDF)
27 536 [lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1991L0414:20070201:EN:PDF](http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1991L0414:20070201:EN:PDF)
28 537 Document No. SANCO/2007/3131 Method validation and quality control procedures for
29 538 pesticide residues analysis in food and feed. Available from:
30 539 http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf
31
32 540 European Committee of Standardization (CEN) Standard method EN 15622. 2008.
33
34 541 Fernández-Alba A R, García-Reyes J F. 2008. Large-scale multi-residue methods for
35 542 pesticides and their degradation products in food by advanced LC-MS. TrAC 27(11) 973-990.
36 543 Granby K, Andersen J H, Christensen H B. 2004. Analysis of pesticides in fruit, vegetables
37 544 and cereals using methanolic extraction and detection by liquid chromatography–tandem mass
38 545 spectrometry. Anal. Chim. Acta 520 165–176.
39
40 546 Hernández F, Pozo O J, Sancho J V, Bijlsma L, Barreda M, Pitarch E. 2006. Multiresidue
41 547 liquid chromatography tandem mass spectrometry determination of 52 non gas
42 548 chromatography-amenable pesticides and metabolites in different food commodities. Journal
43 549 of Chrom. A 1109 242–252.
44
45 550 Kmellár B, Fodor P, Pareja L, Ferrer C, Martínez-Uroz M A, Valverde A, Fernandez-Alba A
46 551 R. 2008. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in

- 1
2
3 552 multi-class vegetables by liquid chromatography–tandem mass spectrometry. *Journal of*
4 553 *Chrom. A* 1215 37–50.
5
6 554 Kruve A, Kunnapas A, HerodesK, Leito I. 2008. Matrix effects in pesticide multi-residue
7 555 analysis by liquid chromatography–mass spectrometry. *Journal of Chrom. A* 1187 58–66.
8
9 556 Lehotay S J, De Kok A, Hiemstra M, Van Bodegraven P. 2005. Validation of a Fast and Easy
10 557 Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using
11 558 Gas and Liquid Chromatography and Mass Spectrometric Detection. *Journal of AOAC Int.*
12 559 88(2) 595-614.
13
14 560 Lehotay S J, Mastovska K, Amirav A, Fialkov A B, Alon T, Martos P A, de Kok A,
15 561 Fernández-Alba A R. 2008. Identification and confirmation of chemical residues in food by
16 562 chromatography-mass spectrometry and other techniques. *TrAC* 27(11) 1070-1090.
17
18 563 Lehotay S J. 2007. Determination of Pesticide Residues in Foods by Acetonitrile Extraction
19 564 and Partitioning with Magnesium Sulfate: Collaborative Study. *Journal of AOAC Int.* 90(2)
20 565 485-520.
21
22 566 Lehotay S J, Mastovská K, Lightfield A R. 2005. Use of Buffering and Other Means to
23 567 Improve Results of Problematic Pesticides in a Fast and Easy Method for Residue Analysis of
24 568 Fruits and Vegetables *Journal of AOAC Int.* 88(2) 615-629.
25
26 569 Milman B L. 2005. Identification of chemical compounds. *TrAC* 24(6) 493-508.
27
28 570 Niessen W M A, Manini P, Andreoli R. 2006. Matrix effects in quantitative pesticide analysis
29 571 using liquid chromatography–mass spectrometry. *Mass Spectrom. Rev.* 25 881– 899.
30
31 572 Ortelli D, Edder P, Corvi C. 2004. Multiresidue analysis of 74 pesticides in fruits and
32 573 vegetables by liquid chromatography–electrospray–tandem mass spectrometry. *Anal. Chim.*
33 574 *Acta* 520 33–45.
34
35 575 Pizzutti I R, de Kok A, Zanella R, Adaime M B, Hiemstra M, Wickert C, Prestes O D. 2007.
36 576 Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid
37 577 chromatography–tandem mass spectrometry using positive and negative electrospray
38 578 ionization. *Journal of Chrom. A* 1142 123–136.
39
40 579 Pozo O J, Barreda M, Sancho J V, Hernández F, Lliberia J L, Cortés M A, Bagó B. 2007.
41 580 Multiresidue pesticide analysis of fruits by ultra-performance liquid chromatography tandem
42 581 mass spectrometry. *Anal. Bioanal. Chem.* 389 1765–1771.
43
44 582
45
46
47
48
49
50
51
52
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Table 1. Detection parameters in the MRM quantitative method for 55 pesticides plus the TPP surrogate standard, LOD of the MRM and EPI screening method. (DP: Declustering Potential; EP: Entrance Potential; CEP: Collision Cell Entrance Potential; CE: Collision Energy; CXP: Collision Cell Exit Potential)

pesticide	MW (amu)	[M+H] ⁺ (m/z)	product ions (m/z)	ion ratio (%)	DP	EP	CEP	CE	CXP	t _R (min)	pk width (min)	MRM LOD (ng/kg)	EPI LOD (ng/kg)
Acetamiprid	222.1	223.2	126.1 56.1	22	26	4.5	14	25 33	4 4	11.5	1.02	4	10
Azoxystrobin	403.4	404.0	372.0 329.0	33	31	5.5	18	19 39	12 4	22.0	1.03	1	4
Boscalid	343.2	343.0	307.0 140.0	61	56	8.5	16	25 25	10 4	22.3	0.81	4	40
Bupirimate	316.1	317.2	108.0 166.2	86	26	8.5	34	37 31	2 4	20.1	1.41	1	4
Buprofezin	305.4	306.0	201.0 116.0	93	31	4	16	17 21	4 4	25.7	1.56	1	10
Carbaryl	201.2	202.1	145.0 127.0	34	26	6	14	13 35	4 4	17.8	1.31	1	20
Carbendazim	191.2	192.2	160.0 132.0	17	36	8.5	12	23 39	4 4	3.6	1.45	1	4
Cyprodinil	225.1	226.2	76.9 93.1	80	41	4	16	55 43	4 8	19.4	1.69	4	10
Diazinon	304.3	305.0	169.0 153.0	62	36	5	16	31 25	4 4	26.5	1.29	1	1
Difenoconazole	406.3	406.1	251.0 111.0	32	41	6.5	30	31 73	4 4	24.9	1.06	1	10
Diflubenzuron	310.7	311.0	158.1 141.1	15	71	10. 5	14	19 39	4 4	23.2	0.64	20	40
Dimethoate	229.3	230.0	125.0 199.0	97	16	4.5	14	25 13	4 4	11.3	1.01	1	20
Dimethomorph	387.0	388.2	301.2 165.2	58	51	4.5	20	27 43	4 4	19.5 & 20.0	1.60	1	40

pesticide	MW (amu)	[M+H] ⁺ (m/z)	product ions (m/z)	ion ratio (%)	DP	EP	CEP	CE	CXP	t _R (min)	pk width (min)	MRM LOD (ng/kg)	EPI LOD (ng/kg)
Diphenylamine	169.2	170.0	93.0 66.0	17	51	8	12	31 59	4 4	24.3	1.23	<1	<1
Fenamiphos	303.4	304.0	217.0 234.0	59	41	4	14	29 21	4 4	21.7	1.25	1	4
Fenzaquin	306.4	307.0	57.0 161.0	80	41	4	14	39 21	4 4	29.4	1.62	1	10
Fenhexamid	302.0	302.1	97.1 55.0	63	51	4	16	33 59	4 6	22.2	1.28	1	20
Haloxfop	361.0	362.1	316.1	*	61	7.5	16	23	8	23.5	0.78	10	
Hexythiazox	352.0	353.2	228.0 168.1	81	41	4.5	16	21 33	4 4	30.0	1.21	1	20
Imazalil	297.2	297.0	159.0 201.0	88	46	4	14	31 23	4 4	14.3	1.69	1	10
Imidacloprid	255.7	256.0	209.0 175.0	84	31	4.5	16	19 23	6 4	10.5	0.90	4	40
Indoxacarb	527.8	528.0	249.0 150.0	84	66	4	22	23 31	4 4	27.6	0.98	1	40
Isofenphos	345.4	346.0	217.0 245.0	34	16	2.5	14	29 17	6 8	28.1	0.62	1	40
Isofenphos- methyl	331.4	332.2	273.0 231.0	79	21	2.5	14	11 17	6 8	26.7	0.75	4	-
Kresoxim-methyl	313.4	314.0	206.0 116.0	59	21	9	14	11 19	4 4	25.4	1.14	20	40
Linuron	248.0	249.0	182.0 160.0	97	41	10	16	21 25	4 4	21.5	0.83	10	20
Malathion	330.4	331.1	127.0 99.1	86	26	8	16	17 29	4 4	24.1	0.84	1	40
Metalaxyl	279.3	280.0	220.0 192.0	85	31	4.5	14	17 23	4 4	18.1	1.09	1	10
Methamidophos	141.1	142.0	94.0	92	31	4.5	10	17	4	3.2	0.96	4	20

pesticide	MW (amu)	[M+H] ⁺ (m/z)	product ions (m/z)	ion ratio (%)	DP	EP	CEP	CE	CXP	t _R (min)	pk width (min)	MRM LOD (ng/kg)	EPI LOD (ng/kg)
			125.0					17	4				
Methiocarb	225.3	226.0	169.0	73	26	9.5	14	13	4	21.1	1.20	1	-
			121.0					23	4				
Methiocarb- sulfone	257.3	258.1	122.1	69	36	5	16	21	4	12.7	0.98	-	-
			201.1					13	4				
Methiocarb- sulfoxide	241.3	242.2	185.0	35	31	4.5	14	17	4	8.4	1.37	4	20
			122.0					37	4				
Methomyl	162.2	163.1	106.0	94	21	4	10	13	4	6.5	1.64	4	40
			88.0					13	4				
Oxamyl	219.3	220.0	72.0	**	21	2	14	15	4	5.3	1.21	-	-
			90.0					13	4				
Penconazole	284.2	286.0	160.9	67	31	4.5	20	35	4	23.5	1.08	1	4
			70.0					31	4				
Pendimethalin	281.3	282.0	212.0	19	21	4	16	15	6	29.9	1.03	10	40
			194.0					23	4				
Phosalone	367.8	368.0	182.0	31	41	9.5	20	19	4	27.1	0.82	10	20
			111.0					53	4				
Phosmet	317.3	318.0	160.0	13	26	8.5	14	17	4	22.0	1.01	10	40
			133.0					49	4				
Pirimicarb	238.0	239.2	182.2	93	36	4.5	16	21	4	6.9	1.87	1	10
			72.0					31	4				
Pirimiphos- methyl	305.0	306.2	164.3	68	51	4.5	22	29	4	26.5	1.32	1	10
			108.1					41	4				
Prochloraz	376.7	378.1	310.1	37	21	4.5	24	17	6	20.2	1.16	1	20
			268.0					21	8				
Propamocarb	188.3	189.0	102.0	39	31	6	12	23	4	3.2	1.25	1	10
			144.0					17	4				
Propargite	350.5	368.2***	231.2	65	36	5.5	16	15	4	30.7	0.67	4	20
			175.3					19	4				
Pymetrozine	217.2	218.1	105.0	11	51	9	14	25	4	2.5	1.11	10	20

pesticide	MW (amu)	[M+H] ⁺ (m/z)	product ions (m/z)	ion ratio (%)	DP	EP	CEP	CE	CXP	t _R (min)	pk width (min)	MRM LOD (ng/kg)	EPI LOD (ng/kg)
			78.1					55	4				
Pyraclostrobin	387.8	388.0	194.0 163.0	98	26	5	16	17 29	6 4	26.0	0.99	1	10
Pyrimethanil	199.3	200.2	107.1 82.1	62	46	7.5	12	31 35	4 4	15.9	1.47	1	10
Pyriproxyfen	321.4	322.0	96.0 185.0	62	31	2.5	20	21 29	4 4	28.9	1.48	1	4
Quinoxifen	308.1	308.0	197.0 162.0	97	61	4	14	43 61	4 4	27.3	1.26	1	20
Tebuconazole	307.8	308.1	70.0 125.0	55	41	4.5	14	41 45	4 4	22.6	1.26	1	10
Thiabendazole	201.3	202.0	175.0 131.0	84	56	6.5	14	35 43	4 4	3.8	1.66	1	4
Thiacloprid	252.7	253.0	126.0 99.0	18	51	4	16	27 57	4 4	13.6	1.55	4	20
Thiamethoxam	291.0	292.1	211.2 181.1	39	26	9.5	16	17 29	4 4	7.9	1.23	10	40
Thiophanate- methyl	342.4	343.1	151.1 311.1	14	26	6.5	18	25 17	4 10	15.8	1.04	4	20
TPP	326.0	327.0	152.0 215.0	78	61	12		43 33	4 4	25.9	1.07	1	-
Triadimenol	295.8	296.0	70.0 227.0	9	21	4	14	21 13	4 4	20.3 & 20.8	1.76	10	40
Trifloxystrobin	408.4	409.2	186.1 206.2	37	26	7	18	23 19	4 4	27.9	1.25	1	4

* no second ion; **insensitive signal; *** [M+NH₄]⁺ (M+18)

Table 2. Average least linear squared calibration line slopes for the pesticide analytes (listed in order of increasing t_R) normalized to the equivalent 0.333 mg/kg TPP internal standard signal ($*10^{-3}$) in matrix-matched calibration standards for 12 different matrices (n = 10): 1 = tomato; 2 = apple; 3 = lettuce; 4 = cucumber; 5 = carrot; 6 = mushroom; 7 = grape; 8 = lemon; 9 = pepper; 10 = pear; 11 = potato; 12 = cabbage. Bold values indicate RSD > 20%.

Pesticide	Commodity												Overall Ave. ± SD, n = 12
	1	2	3	4	5	6	7	8	9	10	11	12	
Pymetrozine	8.2	15.7	8.3	9.4	12.1	8.0	11.5	6.0	8.8	12.2	8.1	7.3	9.6 ± 2.6
Methamidophos	4.0	4.1	3.8	4.5	3.3	4.9	4.1	3.6	3.3	4.1	3.4	3.1	3.8 ± 0.5
Propamocarb	13.6	15.4	13.6	14.0	14.3	15.6	15.0	14.7	12.2	13.8	12.6	12.7	14.0 ± 1.0
Carbendazim	16.7	18.5	17.1	17.2	16.8	19.9	17.3	15.1	14.9	16.3	15.2	15.3	16.7 ± 1.4
Thiabendazole	7.6	8.7	7.8	9.1	8.5	8.6	7.9	7.7	6.6	6.9	6.7	6.7	7.7 ± 0.8
Methomyl	5.2	5.5	5.2	4.6	5.1	6.5	5.6	4.4	4.4	4.9	4.8	4.7	5.1 ± 0.6
Pirimicarb	14.2	14.8	13.1	16.1	13.4	17.1	15.5	13.4	12.6	14.0	11.0	11.9	13.9 ± 1.7
Thiamethoxam	1.7	1.9	1.7	1.4	1.8	2.0	1.6	1.4	1.5	1.8	1.5	1.4	1.6 ± 0.2
Methiocarb-sulfoxide	7.0	7.8	8.4	8.5	13.6	11.6	9.8	7.0	8.2	10.9	9.0	9.6	9.3 ± 1.9
Imidacloprid	1.9	2.5	2.3	1.7	2.6	2.6	2.2	1.8	1.7	2.0	2.2	2.1	2.1 ± 0.3
Dimethoate	6.5	7.1	6.6	6.4	6.6	8.5	7.0	5.8	5.4	5.8	5.3	6.5	6.5 ± 0.8
Acetamiprid	6.4	7.4	7.1	6.9	7.6	8.1	6.9	6.1	5.6	6.1	6.0	6.3	6.7 ± 0.7
Methiocarb-sulfone	0.9	1.6	1.2	1.7	5.3	1.9	2.9	1.8	1.5	2.2	2.0	2.9	2.2 ± 1.1
Thiacloprid	8.6	10.5	9.8	9.2	11.2	10.8	8.6	7.6	7.0	8.1	8.9	9.6	9.2 ± 1.2
Imazalil	3.9	4.4	3.7	4.2	2.9	4.7	4.4	4.3	3.4	3.8	3.2	3.3	3.8 ± 0.5
Thiophanate-methyl	8.2	8.6	7.4	7.3	8.5	8.7	8.9	7.3	6.7	7.6	7.4	7.9	7.9 ± 0.7
Pyrimethanil	6.6	7.6	6.5	7.7	7.1	7.8	7.0	7.1	5.9	6.0	5.8	6.0	6.8 ± 0.7
Carbaryl	14.3	15.6	14.7	12.0	17.7	18.1	15.8	10.6	12.4	13.3	11.4	15.2	14.3 ± 2.3
Metalaxyl	15.1	17.7	16.0	16.4	16.3	18.5	17.2	17.4	13.0	13.7	14.1	16.1	16.0 ± 1.6
Cyprodinil	3.7	3.8	3.3	3.9	3.1	3.5	4.1	3.6	3.4	3.7	2.8	3.1	3.5 ± 0.4
Dimethomorph	4.9	5.6	5.3	4.9	5.9	6.1	5.5	5.5	4.3	4.7	5.0	5.4	5.3 ± 0.5
Bupirimate	4.9	4.9	4.2	5.2	4.1	5.4	5.3	5.3	4.4	4.5	3.8	4.2	4.7 ± 0.5
Prochloraz	6.1	7.0	5.8	5.5	4.5	7.4	7.0	5.3	5.6	5.6	5.7	6.1	6.0 ± 0.8
Triadimenol	7.2	8.4	6.5	7.1	9.5	11.0	9.3	7.7	6.6	7.8	6.1	7.2	7.9 ± 1.4
Methiocarb	8.8	10.5	9.9	7.9	11.3	11.8	10.3	9.0	8.3	8.8	9.6	10.5	9.7 ± 1.2

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3	Linuron	2.8	4.1	2.7	2.8	3.1	2.9	2.9	3.0	2.0	3.1	2.7	2.8	2.9 ± 0.4
4	Fenamiphos	16.5	19.0	16.8	16.9	16.8	19.3	18.6	18.5	14.0	16.1	14.5	17.2	17.0 ± 1.6
5	Azoxystrobin	17.4	18.2	16.9	16.2	17.7	19.7	19.4	17.9	15.8	16.4	16.2	19.2	17.6 ± 1.3
6	Phosmet	4.7	5.3	5.5	4.0	7.3	7.2	6.6	3.2	4.9	6.1	5.8	6.2	5.6 ± 1.2
7	Fenhexamid	4.8	5.9	5.4	5.0	5.3	5.8	5.8	4.9	4.3	5.2	5.0	5.1	5.2 ± 0.4
8	Boscalid	2.5	3.0	2.9	2.4	3.1	3.0	3.0	2.6	2.2	2.6	2.6	2.9	2.7 ± 0.3
9	Tebuconazole	14.8	18.3	15.9	13.5	16.1	18.0	17.9	15.8	14.1	15.6	14.5	14.5	15.8 ± 1.5
10	Diflubenzuron	2.4	3.7	2.5	2.2	3.5	2.9	2.6	2.7	1.9	2.2	2.1	2.4	2.6 ± 0.5
11	Haloxypop	1.0	2.4	1.2	1.1	2.5	2.8	1.0	1.3	0.8	1.1	1.2	1.0	1.4 ± 0.7
12	Penconazole	4.3	8.6	4.9	4.4	7.7	9.2	4.4	5.4	3.3	4.2	4.8	4.0	5.4 ± 1.9
13	Malathion	8.0	8.7	8.3	7.4	9.7	9.8	8.9	7.5	7.3	7.4	7.8	8.9	8.3 ± 0.8
14	Diphenylamine	9.1	11.2	9.8	8.8	9.8	11.4	11.0	9.2	8.3	10.0	9.3	8.4	9.7 ± 1.0
15	Difenoconazole	11.6	13.1	11.2	11.3	13.2	14.2	13.9	10.6	11.4	11.5	11.6	12.0	12.1 ± 1.1
16	Kresoxim-methyl	0.2	0.3	0.2	0.3	0.5	0.4	0.2	0.3	0.2	0.2	0.2	0.2	0.3 ± 0.1
17	Buprofezin	27.4	28.6	24.1	26.9	26.7	26.4	31.7	27.7	25.5	24.9	22.2	25.5	26.5 ± 2.3
18	Pyraclostrobin	11.3	11.2	10.7	10.0	10.9	12.3	12.2	10.6	10.2	10.0	10.0	11.5	10.9 ± 0.8
19	Diazinon	27.6	30.7	25.2	31.6	24.4	31.3	32.6	31.7	26.7	28.2	22.3	23.6	28.0 ± 3.4
20	Pirimiphos-methyl	19.6	20.9	17.4	21.6	18.3	22.3	22.6	20.4	18.7	19.9	16.1	16.7	19.5 ± 2.0
21	Isofenphos-methyl	0.8	1.1	0.4	1.8	1.5	1.9	0.8	1.4	0.8	0.7	0.7	0.8	1.1 ± 0.5
22	Phosalone	3.2	4.2	2.9	3.4	3.2	2.5	3.4	3.1	2.3	3.8	3.4	2.4	3.2 ± 0.5
23	Quinoxifen	5.3	4.8	4.4	4.3	4.2	4.3	5.8	4.1	4.7	5.2	4.1	4.3	4.6 ± 0.5
24	Indoxacarb	0.9	1.0	0.9	0.8	1.0	1.0	1.1	0.8	0.8	1.0	0.9	1.1	0.9 ± 0.1
25	Trifloxystrobin	15.0	12.5	12.2	14.8	11.6	13.8	16.0	13.0	12.9	13.2	11.4	13.4	13.3 ± 1.3
26	Isofenphos	0.4	0.5	0.2	0.7	0.6	0.8	0.4	0.9	0.5	0.2	0.3	0.3	0.5 ± 0.2
27	Pyriproxyfen	26.2	20.4	19.6	22.9	21.1	20.0	27.9	20.1	22.9	26.6	17.8	21.5	22.2 ± 3.0
28	Fenazaquin	16.7	13.3	11.7	15.8	11.8	12.7	16.9	13.4	15.4	16.2	11.0	11.8	13.9 ± 2.1
29	Pendimethalin	2.4	1.8	1.8	2.0	1.8	1.8	2.7	1.8	1.9	2.2	1.8	2.3	2.0 ± 0.3
30	Hexythiazox	2.7	1.8	2.0	2.4	1.8	1.8	2.7	1.7	2.0	2.3	1.9	2.2	2.1 ± 0.3
31	Propargite	0.8	0.3	0.5	1.0	0.4	0.4	0.8	0.7	0.6	0.5	0.3	0.7	0.6 ± 0.2
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Table 3. Distribution of total and samples found to contain residues per commodity analyzed.

	Commodity	No. positive / total samples
Fruits	Apple	7/14
	Grapes	6/9
	Lemon	8/8
	Watermelon	2/8
	Pear	5/6
	Peach	3/6
	Banana	5/5
	Nectarine	3/3
	Pineapple	0/2
	Strawberry	2/2
	Grapefruit	2/2
	Orange	2/2
	Melon	0/2
Vegetables	Pepper	10/23
	Lettuce	10/18
	Tomato	5/15
	Cabbage	7/16
	Carrot	5/6
	Garlic	2/5
	Cucumber	4/5
	Parsnip	1/5
	Onion	0/5
	Celery	2/3
	Radish	0/2
Pumpkin	0/2	
Other	Mushroom	3/8
	Misc.	6/9
Total		100/200

Table 4. Determined pesticides and their concentration ranges in the 200 samples analyzed.

Pesticide	Fruits (71 samples)			Vegetables/Other (129 samples)		
	No. of positive samples	Min - Max (mg/kg)	No. of violations	No. of positive samples	Min - Max (mg/kg)	No. of violations
Fungicides						
Azoxystrobin	1	0.109		14	0.010-0.551	
Boscalid	2	0.017-0.029		1	10.4	1
Bupirimate	2	0.005-0.008		-	-	
Carbendazim	4	0.041-0.064		4	0.041-0.352	2
Cyprodinil	7	0.010-0.364		-	-	
Diethofencarb	-	-		1	0.030	
Difenoconazole	1	0.025		-	-	
Dimethomorph	-	-		1	0.176	1
Diphenylamine	16	0.007-0.081	1	29	0.006-0.202	4
Fenhexamid	1	0.189		-	-	
Imazalil	15	0.030-6.655		-	-	
Metalaxyl	2	0.087-0.111		2	0.032-0.060	
Myclobutanyl	1	0.102		-	-	
Prochloraz	3	1.045-5.043		-	-	
Propamocarb	-	-		5	0.100-0.475	
Pyraclostrobin	1	0.069		1	0.230	
Pyrimethanil	2	0.010-0.039		-	-	
Quinoxifen	2	0.010-0.012		-	-	
Spiroxamine	1	0.189		-	-	
Tebuconazole	-	-		1	0.040	
Thiabendazole	10	0.035-0.899		-	-	
Triadimenol	1	0.018		-	-	
Herbicides						
Haloxifop	-	-		1	0.637	1
Linuron	-	-		1	0.314	
Insecticides						
Acetamiprid	1	0.035		3	0.037-0.098	
Buprofezin	-	-		1	0.043	
Diflubenzuron	4	0.020-0.127		-	-	
Diazinon	2	0.020-0.029	1	-	-	
Dimethoate	-	-		1	0.244	1
Indoxacarb	-	-		1	0.054	
Phosmet	2	0.370-0.447		-	-	
Pirimicarb	2	0.022-0.024		-	-	
Pymetrozine	-	-		1	0.199	
Pyriproxyfen	-	-		1	0.010	
Thiamethoxam	-	-		2	0.111-0.290	

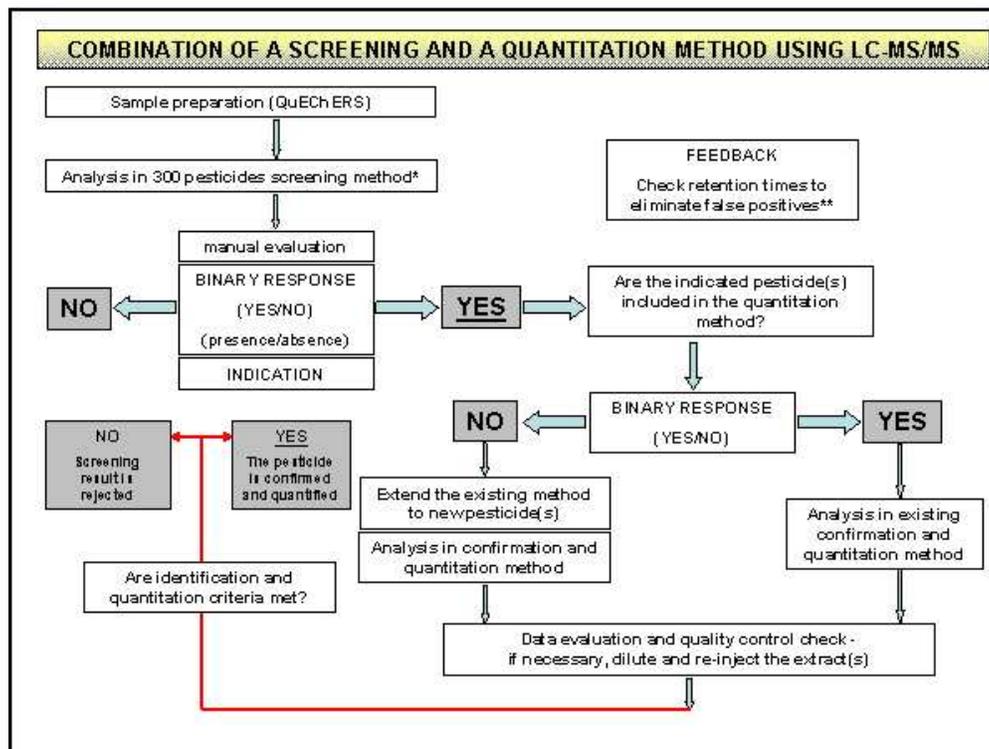


Figure 1. Workflow for the combination of a screening and quantitation approach by LC-MS/MS. For details, see text. *: commercial method of Applied Biosystems; **: the retention times of the appropriate ion peaks have to be the same for the analyte in both methods

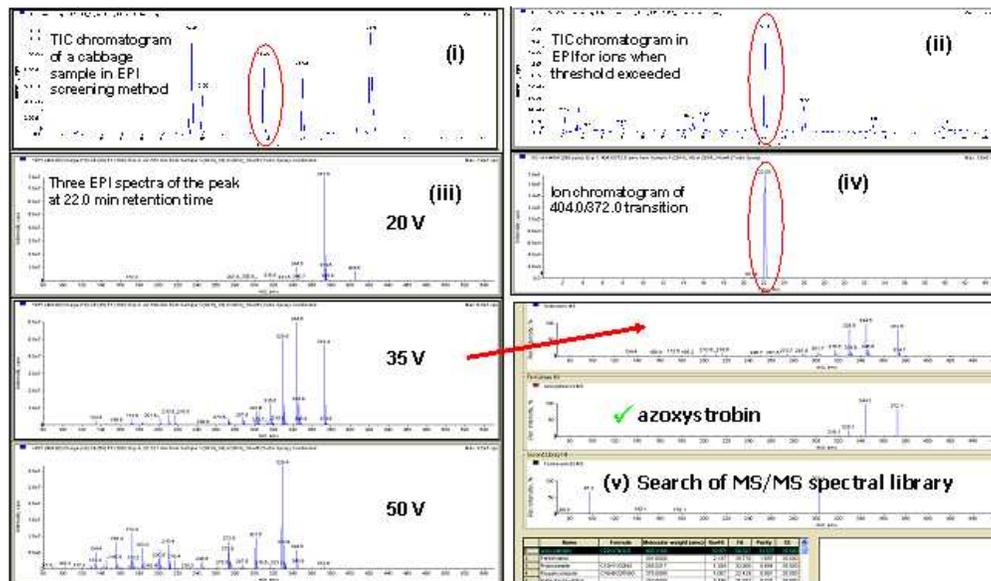


Figure 2. Example of the MRM-triggered EPI screening identification process: azoxystrobin was indicated in a cabbage extract at the retention time of 22.0 min using the pesticide MS/MS library of 300 pesticides (for details, see text).
170x100mm (96 x 96 DPI)

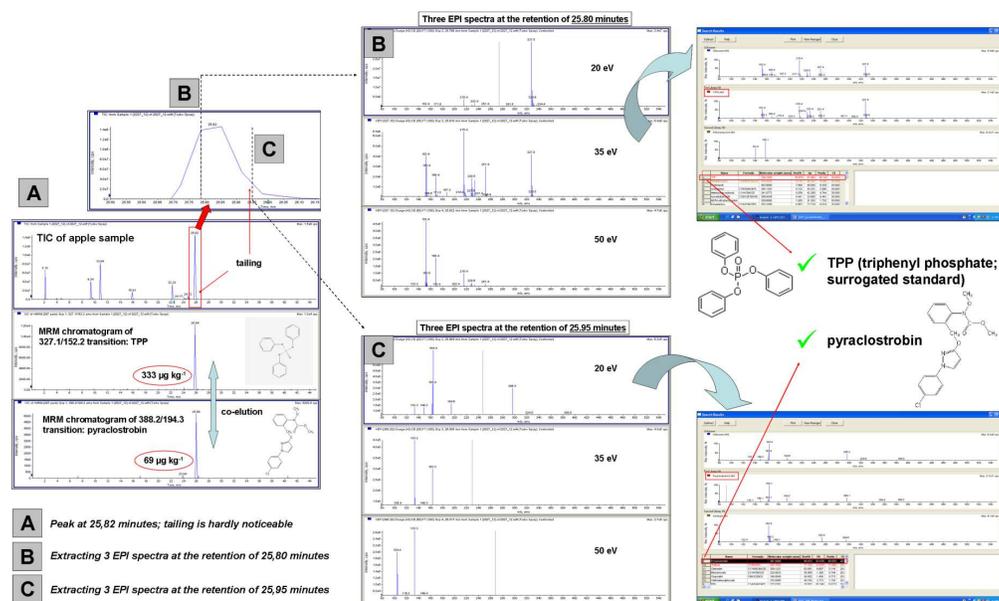


Figure 3. Careful data review was needed due to co-elution of TPP and pyraclostrobin: a) TIC and the MRM chromatograms of TPP and pyraclostrobin; b) EPI spectra extracted from the peak apex (25.80 min) and identification of TPP (added at 333 mg kg⁻¹ into the sample); and c) EPI spectra extracted from the tail of the peak (25.95 min) and identification of pyraclostrobin.
487x291mm (96 x 96 DPI)

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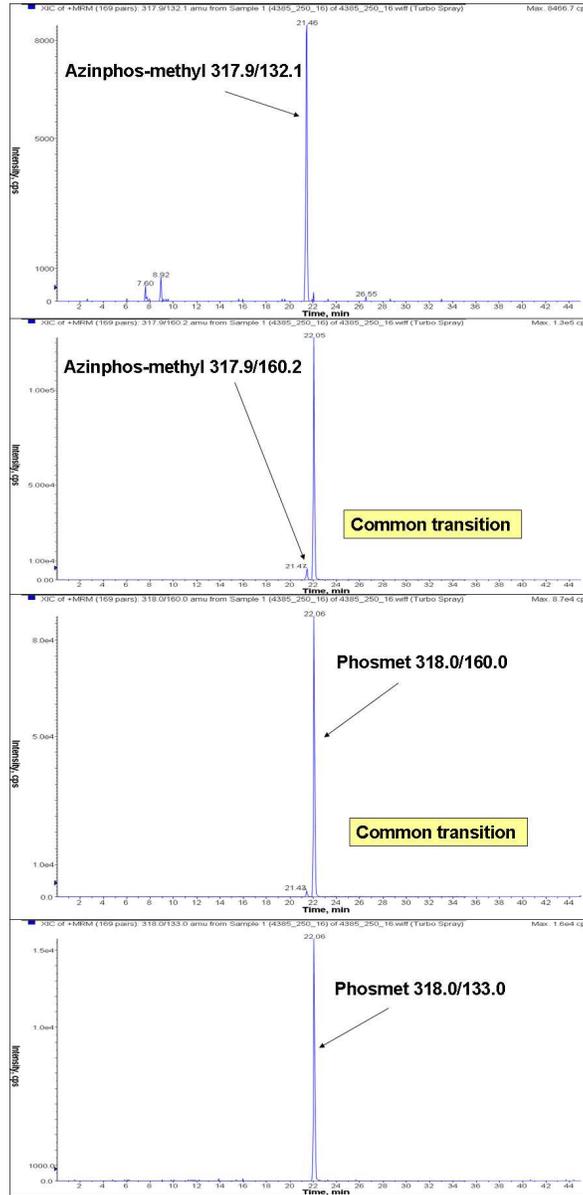


Figure 4. Situation for similar ion transitions and retention times of azinphos-methyl and phosmet.
237x484mm (96 x 96 DPI)

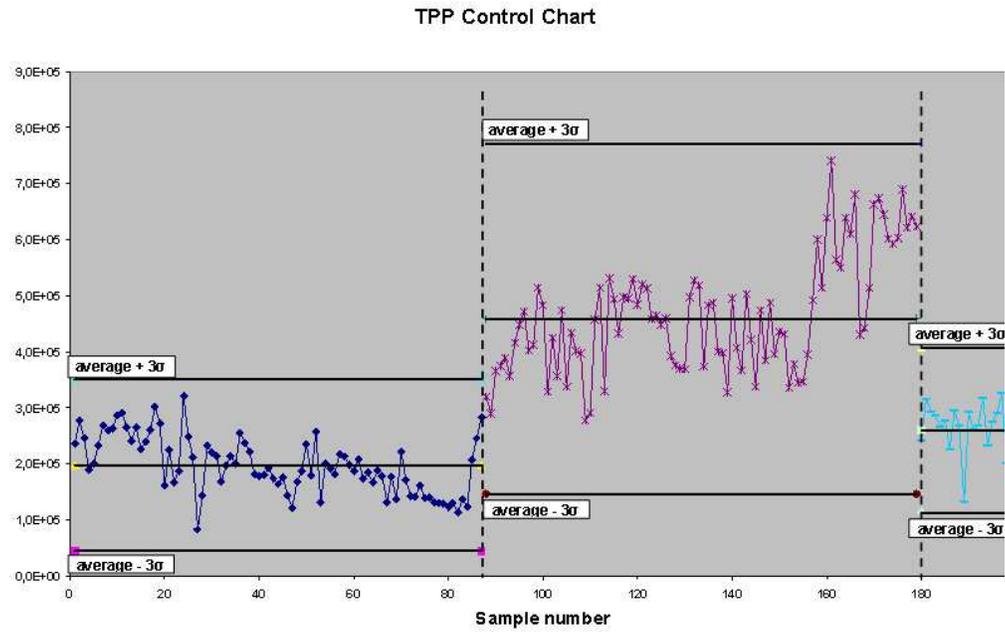


Figure 5. Quality control chart of 0.333 mg kg⁻¹ equivalent concentration of triphenyl phosphate (TPP) as the surrogate standard in 3 groups of results for the 200 tested samples.

Review Only