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Development of an LC-MS/MS method for the determination of pesticides and patulin in apples.

Abstract

A method for the simultaneous determination of 33 pesticides or degradation products together with patulin in apples by LC-MS/MS was developed. The method involved homogenisation of the apples, extraction with ammonium acetate–acetic acid solution in methanol–water by ultrasonication, filtration and determination by LC-MS/MS. The repeatability and within-laboratory reproducibility for the three spiking levels 0.02, 0.04 and 0.2 mg kg⁻¹ were between 4 and 35%. In general, the repeatability and reproducibility were about 10–20%. The limits of quantification (LOQs) were between 0.01–0.14 mg kg⁻¹. The method was used on incurred samples from parts of the ISAFRUIT project financed by the European Commission under the 6th Framework Programme. Samples were analysed at four different stages, after harvest, after storage (controlled), after water bath and after 28 days at room temperature. Pesticide residues were found at all stages, but no significant differences in the concentration were seen between the stages analysed. The concentration decreased significantly only for tolylfluanid after storage at room temperature for 28 days when only 0–6% of the original amount of tolylfluanid remained in the apples. No patulin was found in the apples stored for 28 days at room temperature and no growth of *P. expansum* was observed on these apples. However, when the apples were inoculated with a spore suspension of *P. expansum* high concentrations of patulin were found.

Keywords: apples, pesticides, patulin, *Penicillium expansum*, LC-MS/MS.

Introduction

Pesticides are widely used in the production of apples, both pre- and post-harvest to protect the apples from a range of pests and fungi, but also to preserve quality. Food Authorities such as the European Commission (EC) have established Maximum Residue Levels (MRLs) for the majority of the pesticides used in the production of apples (European Community, 2006). Monitoring programmes for pesticides have shown that fungicides and insecticides are often found in apples. In 59% of apple samples analysed in the EC coordinated monitoring programme for 2004 pesticide residues were found (European Commission, 2004). Although, residues above the MRLs were

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2 found only in 2% of the samples, many consumers are nevertheless concerned about exposure to
3 pesticide residues.
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7 Several fungi can produce mycotoxins either in the field or during storage, and especially for apples
8 *Penicillium expansum* is by far the most dominant fungal species. *P. expansum* is a known producer
9 of patulin, and the European Communities (EC), have introduced a maximum limit of 50 µg kg⁻¹,
10 for patulin content in apple juice and other apple products (European Commission, 2003).
11 Furthermore, *P. expansum* spoilage of and hence patulin content in apples is a well known problem
12 for the apple juice industry as apples are commonly stored prior to production due to processing
13 capacity limitations. Patulin is therefore often used as an indicator of the use of poor quality raw
14 materials in juice manufacture (Pitt, 2002). The analytical methods for the determination of patulin
15 in fruit and vegetables have often been carried out by methods dedicated to a single analyte, where
16 HPLC with UV detection is commonly employed (Iha and Sabino, 2006; MacDonald *et al.*, 2000).
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27 Commonly, pesticide residues have been detected by so called multi-residue methods that cover
28 several hundred compounds. However, risk assessment involving different contaminant groups have
29 necessitated multi-residue methods covering different compound groups. No previous published
30 papers have, to the authors knowledge, described the simultaneously determination of pesticides
31 and mycotoxins in apples.
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37 In the present study an integrated method for sample preparation, extraction and determination was
38 developed for the simultaneous analysis of several pesticides together with patulin. The method was
39 used especially, to identify the critical steps for chemical contaminants in the chain from orchard to
40 consumer. Results from apples analysed after harvest, storage, grading and after shelf life are
41 presented. The study was part of the European Integrated Research Project, ISAFRUIT that focuses
42 on all aspects of fruit. It was financed via the 6th Framework Programme (Thematic Priority 5 -
43 Food Quality and Safety). For more information on the project see the website, www.isafruit.org
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51 **Materials and Methods**

52 ***Field trials***

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54 An experiment with the apple variety *Malus Domestica* (Golden delicious) to simulate the chain
55 from orchard to consumers was performed at Agroscope Wädenswil (Switzerland). The apples
56 were treated with the fungicides, trifloxystrobin, flusilazole, pirimicarb and thiacloprid at different
57 application times (see Table 1). For the fungicide tolylfluanid four different application times were
58 used in order to achieve different concentrations of the residue. Samples were collected at four
59
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1
2 critical points to simulate the chain from producer to consumer. The four points were 1) after
3 harvest, 2) after storage for 6 month at 4°C in controlled atmosphere with low oxygen
4 concentration, 3) after grading (here represented as a warm water bath at 30°C for 10 min)
5 performed the day that the apples were taken out of storage and 4) after 28 days shelf life at room
6 temperature to simulate the consumers table.
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12 [insert table 1]
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16 Samples of 1 kg and at least 10 apples were collected from each group (1-4) and send to the
17 laboratory at the National Food Institute, Technical University of Denmark, Denmark. Analysis was
18 performed on samples collected at the four above mentioned critical point in the chain. See table 2.
19 The samples were packed in polyethylene bags and transported as quickly as possible to avoid any
20 degradation during transportation.
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27 [insert table 2]
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30 *Incubation of apples with P. expansum*

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32 The *P. expansum* isolate, IBT number 23732, the type culture used in this study, was obtained from
33 the Fungal Culture Collection (IBT collection) at the Department of Systems Biology, Technical
34 University of Denmark, Denmark. The apples were divided into three groups of 10 apples
35 (replicates). Group A was untreated, group B was surface inoculated with *P. expansum* and group C
36 was inoculated in the core with *P. expansum*. The surface inoculated apples were stab wounded
37 with a sterilised needle (4-5 mm wound) and 10 µl of spore suspension ($>10^6$ spores ml⁻¹) was put
38 on the wound. Core inoculated apples were inoculated through the flower opening via a syringe and
39 10 µl of spore suspension ($> 10^6$ spores ml⁻¹) was injected. The apples were incubated at room
40 temperature for 28 days.
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49 **Materials and instruments**

50 *Chemicals*

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52 The pesticide standards (with certified purities >96%) were purchased from Dr. Ehrenstorfer
53 (Augsburg, Germany) and the ¹³C₆-carbaryl (purity 99%) from Cambridge Isotope Labs. (MA,
54 USA). The patulin was purchased from Sigma Aldrich (USA). The methanol p.a. was from Fisher
55 Scientific (Leicestershire, UK). Acetic acid and ammonium acetate were from Merck (Darmstadt,
56 Germany). Mini Uniprep polypropylene filter vials 500 µl, pore size 0.45µm was purchased from
57 Whatman (Kent, UK). The water used was Milli-Q water (Millipore, Bedford, MA, USA).
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4 Stock solutions of pesticides and patulin of 1mg ml^{-1} were prepared in methanol and kept at $-18\text{ }^{\circ}\text{C}$.
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6 A standard mixture of $10\text{ }\mu\text{g ml}^{-1}$ in methanol was prepared from the stock solutions. Matrix-
7
8 matched standards in the concentration range from 0.0015 to $0.05\text{ }\mu\text{g ml}^{-1}$ (0.007 – 0.24 mg kg^{-1}
9
10 matrix) were prepared by diluting the $10\text{ }\mu\text{g ml}^{-1}$ mixture with sample extracts of the relevant
11
12 commodities bought as organically grown. The sample extracts were in the extraction solution:
13
14 ammonium acetate / acetic acid 20 mM in methanol–water (95:5), which is the same solution as
15
16 eluent B.

17 18 *Sample preparation*

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20 The apples were homogenised by a chopper (Weisser, Obersasbach, Germany). 10 g of sample was
21
22 extracted with 40 ml ammonium acetate–acetic acid solution 20 mM in methanol–water (95:5) in an
23
24 ultrasonic bath (Branson 5510, Soest, The Netherlands) for 30 minutes. The samples were
25
26 centrifuged at 3500 rpm for 10 min (Heraeus Sepatech Megafuge 3.0R, Osterode, Germany).
27
28 Extracts, $400\text{ }\mu\text{l}$, were transferred to Miniprep filter HPLC vials and added $40\text{ }\mu\text{l}$ internal standard:
29
30 $^{13}\text{C}_6$ -carbaryl $0.1\text{ }\mu\text{g ml}^{-1}$ before filtration.

31 32 *Instrumentation and chromatographic conditions*

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34 The LC system consisted of a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA,
35
36 USA) equipped with a vacuum degasser, a solvent delivery compartment with high pressure
37
38 mixing, an autosampler and a column compartment. The injection volume was $10\text{ }\mu\text{l}$. Separation of
39
40 pesticides was performed on a Genesis C_{18} column, $100\text{ mm} \times 3\text{ mm}$, $4\text{ }\mu\text{m}$ particle size,
41
42 (Gracevydac, Hengoed, UK). In front of the separation column was a Phenomenex SecurityGuard
43
44 column, C_{18} ODS, $4\text{ mm} \times 2\text{ mm}$ (Cheshire, UK). The total flow rate of eluent A (ammonium
45
46 acetate–acetic acid 20 mM in MilliQ water) and B (ammonium acetate–acetic acid 20 mM in
47
48 methanol–MilliQ water (95:5)) was 0.3 ml min^{-1} . The initial gradient was 100% A, decreasing to
49
50 50% A after 2 min and 0% A after 20 min . A was held at 0% until 24 min . The total run time was
51
52 30 min .

53
54 The MS–MS detection was performed on a Quattro Ultima triple quadrupole instrument
55
56 (Micromass Manchester, UK) equipped with an atmospheric pressure ionisation (API) interface.
57
58 The mass spectrometer was operated with electrospray both in the positive and the negative ion
59
60 mode (ESI+ and ESI-). The capillary voltage was set to 1.0 kV . The source temperature was $120\text{ }^{\circ}\text{C}$
and the desolvation temperature $350\text{ }^{\circ}\text{C}$. Nitrogen was used as desolvation gas (flow 550 l h^{-1}) and
cone gas (flow 50 l h^{-1}), and argon was used as collision gas at a pressure of $1.7 \times 10^{-3}\text{ mbar}$.

1
2 Detection was performed in multiple reacting monitoring (MRM) mode. The cone voltage and the
3 collision energy were optimized for the different pesticides and patulin (see Table 3). The
4 quantification was made using Masslynx software with Quanlynx. The maintenance of the mass
5 spectrometer includes cleaning of the cone.
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11 [Insert table 3]
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14 **Results and discussion**

15 *Method Validation*

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17 The pesticides included in the method was chosen due to the possible use on apples. The accuracy
18 of a method can be estimated from certified reference material, recoveries of spiked samples or
19 from accuracy in proficiency testing. Recoveries were obtained by fortifying blank apple samples
20 with pesticides and patulin. The majority of the pesticides, 30, were validated according to
21 Document SANCO/2007/3131 (European Commission, 2007). The mean recoveries of the
22 pesticides (n = 6) at the spiking levels 0.02, 0.04 and 0.2 mg kg⁻¹ appears from Table 4. For most
23 pesticides recoveries were within 70–110%. For the three pesticides trifloxystrobin, tolylfluanid,
24 thiachloprid and for patulin, the determination of the accuracy consisted in recovery test at the three
25 spiking levels, 0.02, 0.04 and 0.2 mg kg⁻¹, single determination with three repetitions (four
26 repetitions for patulin). Again the mean recovery was between 69-109%, see table 5.
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37 [insert table 4]
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40 [inset table 5]
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43 *Precision*

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45 For the 30 pesticides or degradation products, the repeatability and within laboratory reproducibility
46 were calculated according to ISO5725-2 [Anon, 1994] using duplicate determinations for
47 calculations of repeatability and the repetitions for calculation of reproducibility. The repeatability,
48 RSD_r, and reproducibility, RSD_R, for the three spiking levels 0.02, 0.04 and 0.2 mg kg⁻¹ is shown in
49 Table 4. No significant differences were seen between the spiking levels. In general, the
50 repeatability and reproducibility were about 10–20%, which is acceptable for multi-residue
51 methods. For trifloxystrobin, tolylfluanid, thiachloprid the standard deviation between the recovery
52 series were in the range of 4-26% (see table 5).
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Limits of quantification.

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2 The limit of quantification (LOQ) was calculated as 6 times the standard deviation of the absolute
3 recoveries at the lowest accepted spiking level. The LOQ's are given in table 4 for the 46 pesticides,
4 and table 5 for the three pesticides and patulin.
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9 Quantification was performed for each analytical series using means of two 5-point calibration
10 curves. The matrix matched calibrations standards were of the concentrations 0.0015, 0.003, 0.01,
11 0.025 and 0.05 $\mu\text{g ml}^{-1}$. The linearity of the calibration curves were $r^2 > 0.99$. Chromatograms of
12 field treated samples are shown in figure 1.
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19 [insert figure 1]
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25 ***Result from apples sampled along the artificial chain from producer to consumer.***

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28 The validated method was used for the analysis of 64 samples collected from an artificial chain
29 from producer to consumer, described above under Method. Thiacloprid was not found in any of the
30 samples, however this may be due to the fact that thiacloprid was only applied once 90 days before
31 harvest, which may lead to residue content below the detection limit. Patulin was expected only to
32 be present in the samples analysed after 28 days at room temperature and therefore only analysed
33 for in these 16 samples.
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41 No patulin was found in the apple samples analysed after 28 days at room temperature. But to verify
42 the capability of the method, it was used to analyse samples inoculated with *P. expansum*. The
43 results of these samples are presented below.
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47 *Pesticides*

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49 Trifloxystrobin, flusilazole and pirimicarb were found in low concentrations from 0.006-0.024 mg
50 kg^{-1} . These pesticides were applied more than 50 days before harvest and the low levels were
51 expected. No significant differences in the concentration were seen in the samples after harvest,
52 after storage, after grading and samples after 28 days shelf life, see Figure 2. However, due to the
53 low concentrations it may be difficult to see any decrease in the concentration through the chain.
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[insert Figure 2 here]

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2 For tolylfluanid the concentrations were between 0.016-0.488 mg kg⁻¹. The results are shown in
3 Figure 3. The highest concentrations were found in the samples sprayed seven days before harvest.
4 The concentrations found in the samples sprayed 4 weeks before harvest were at the same levels as
5 the tolylfluanid untreated apples. The reasons for the finding of tolylfluanid residues in the
6 untreated samples are supposed to arise from drift of pesticides during spraying. The concentration
7 of the tolylfluanid residue found in the samples collected after harvest were 4-6 times lower than the
8 concentration after storage. According to the Pesticide Manual (Tomlin, 2006), tolylfluanid is
9 rapidly hydrolysed into dimethylamino sulfotoluidide (DMST). The explanation of the low levels in
10 the apples collected after harvest could be that tolylfluanid in the apples were degraded due to
11 exposure to light, heat and moisture during transportation.. These factors were better controlled by
12 wrapping the apples during transportation. The wrapping was consequently improved when next
13 batch of samples were send to the laboratory and these apples arrived in very good condition. No
14 significant decrease in tolylfluanid residues was seen after water bath. However, storage at room
15 temperature in 28 days decreased the concentration significantly. No residues were found in the 28
16 days sample that had been sprayed four and two weeks before harvest. The concentration of
17 tolylfluanid in the samples sprayed 1 week before harvest was 0.03 mg kg⁻¹, which was a reduction
18 of 94%.
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34 [insert Figure 3 here]
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37 The results of pesticide residues from the critical point 2, 3 and 4 are in accordance with an
38 investigation done by Rasmussen et al. (2003) on the effects of washing and storing two different
39 sorts of apples. None of the pesticide included in that study was significantly reduced when the
40 apples were subjected to simple washing. However, storing in a refrigerated room at 4°C for 42-79
41 day significantly reduced 5 out of 13 pesticide residues, including tolylfluanid, which were reduced
42 by 47-69%.
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50 *Patulin*

51 The patulin content and the growth of *P. expansum* in apples treated with pesticides at different
52 times before harvest was investigated after 28 days of shelf-life (see Table 2 for more details).
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57 As shown in Table 6 no patulin was found in the apple samples stored for 28 days at room
58 temperature. Furthermore no growth of *P. expansum* was observed on these apples, showing that
59 patulin producing fungi is not a problem in apple samples stored under the present incubation
60 conditions. When the apples were inoculated by a spore suspension of *P. expansum* patulin had

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been produced at quite high levels in both core and surface inoculations exceeding the EU maximum limit of 50 $\mu\text{g kg}^{-1}$ for apple juice with several hundred percent. In accordance with the study of Karlshøj et al. (2007), the patulin concentrations were 2 – 5 times higher in core inoculated apples compared to the surface inoculated apples. This was probably due to the higher degree of spoilage in these apples as the fungus was able to grow spherically in all directions from the core, whereas the surface inoculated colony was limited to hemispherical growth; that is, the patulin to hyphae ratio between the two types of infection is expected to be the same. It was not possible from the present results to evaluate or explain the effects of pesticide treatment carried at different periods before harvest.

[insert table 6 here]

Conclusions

A method for simultaneous determination of 33 pesticides or degradation products together with patulin in apples was developed. The repeatability and within laboratory reproducibility for the three spiking levels 0.02, 0.04 and 0.2 mg kg^{-1} were between 4-35%. In general, the repeatability and reproducibility were about 10–20%. The limits of detection (LODs) were between 0.01-0.14 mg kg^{-1} . The method was used for the determination of pesticide residues and patulin in samples from the ISAFRUIT project. Trifloxystrobin, flusilazole and pirimicarb were found in low concentrations from 0.006-0.024 mg kg^{-1} . No significant differences in the concentration were seen in the samples after harvest, after storage, after grading and samples after 28 days shelf life. Tolyfluanid was found in concentrations between 0.016-0.488 mg kg^{-1} . No significant decrease in tolyfluanid residues was seen after water bath. However, the storage in room temperature in 28 days decreased the concentration significantly with 94-100%. No patulin was found in the apple samples stored for 28 days at room temperature and no growth of *P. expansum* was observed on these apples. However, patulin were produced in levels exceeding the EU maximum limit of 50 $\mu\text{g kg}^{-1}$ for apple juice, when the apples were inoculated with a spore suspension of *P. expansum*. The patulin concentrations were 2 – 5 times higher in core inoculated apples compared to the surface inoculated apples.

Acknowledgement

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60**Reference:**

Anon, 1994: Accuracy (Trueness and Precision) of Measurements Methods and Results–Part 2 (Geneva: ISO), 1994 (ISO 5725-2:1994).

European Commission Regulation (EC) No 1425/2003. Off J. Eur

European Commission, 2004, Pesticide monitoring reports;
http://ec.europa.eu/food/fvo/specialreports/pesticides_index_en.htm

European Commission, 2006; http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm

European Commission, 2007. Method validation and quality control procedures for pesticide residue analysis in food and feed. SANCO/2007/3131. European Commission.

Granby, K; Andersen, J.H and Christensen, H.B (2004). Analysis of pesticides in fruit, vegetables and cereals using methanolic extraction and detection by liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta* **520**: 164-176.

Iha, M.H. and Sabino, M. (2006). Determination of Patulin in applejuice by liquid chromatography. *Journal of AOAC International*, 89: 139-143.

Karlshøj K, Nielsen PV, Larsen TO. (2007). Prediction of *Penicillium expansum* spoilage and patulin concentration in apples used for apple juice production by electronic nose analysis. *Journal of Agricultural and Food Chemistry* (in press)

MacDonald S, Long M, Gilbert J. (2000). Liquid chromatography method for determination of patulin in clear and cloudy apple juices and apple puree: Collaborative study. *Journal of AOAC International*, 83:1387-1393.

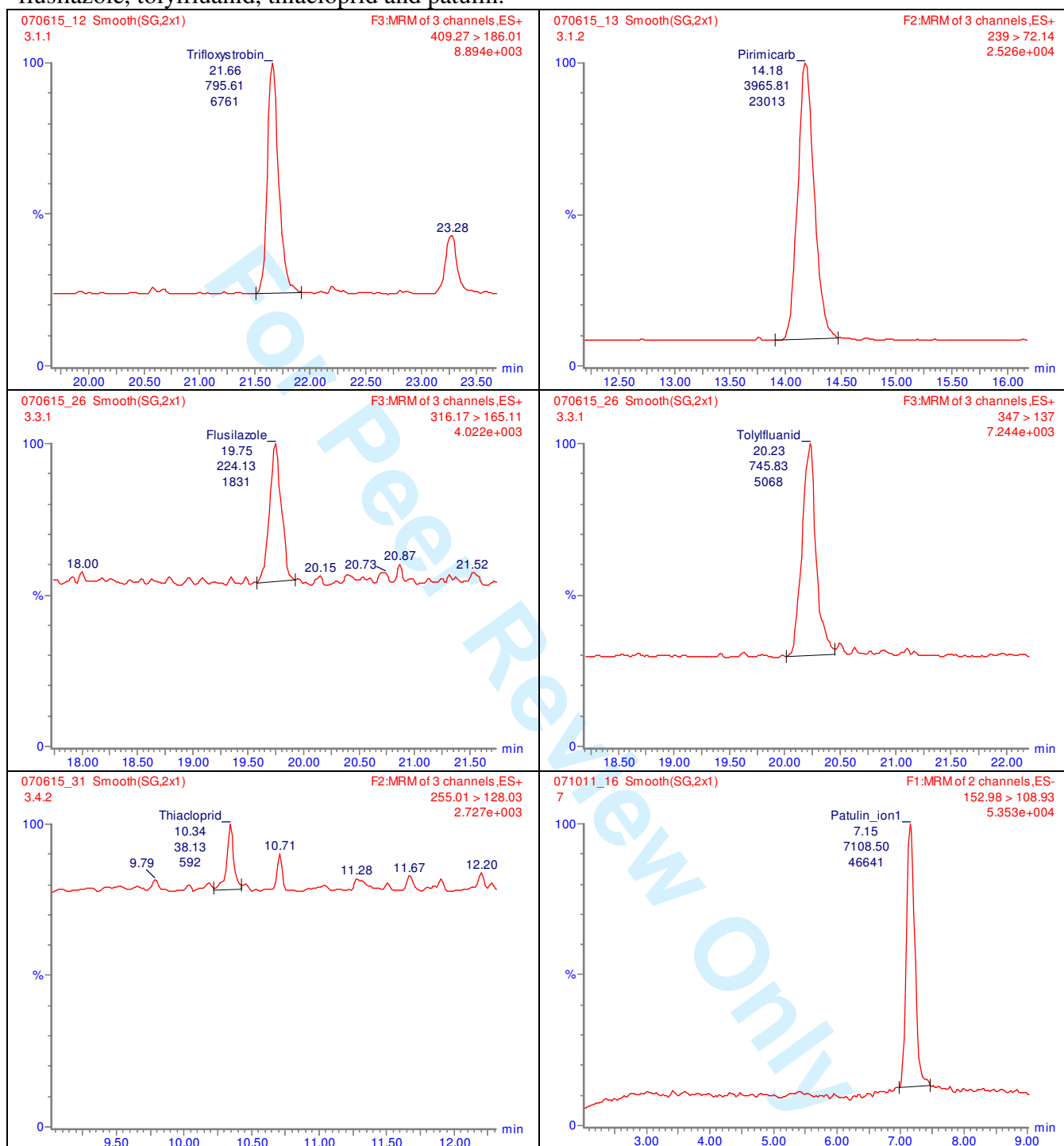
Moukas, A.; Panagiotopoulou, V. and Markaki, P. (2008). Determination of patulin in fruit juices using HPLC-DAD and GC-MSD techniques. *Food Chemistry*, 109: 860-867.

1
2 Pitt J.I. (2002). Biology and ecology of toxigenic *Penicillium* species. In: Mycotoxins and Food
3 Safety, pp 29-41. Eds: DeVries JW, Truckness MW, Jackson LS. Kluwer Academic, New York,
4 USA.
5
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8
9 Rasmussen, R.R., Poulsen, M.E. and Hansen, H.C.B.(2003): Distribution of multiple pesticide
10 residues in apple segments after home processing. *Food Additives and Contaminants*, 20, No. 11,
11 pp. 1044–1063.
12
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16 Tomlin CDS (ed) (2006-2007) The e-Pesticide manual, 14th ed. British Crop Protection Council,
17 London, UK.
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Figure 1. Examples of chromatogrammes of samples containing trifloxystrobin, pirimicarb, flusilazole, tolylfluanid, thiachloprid and patulin.



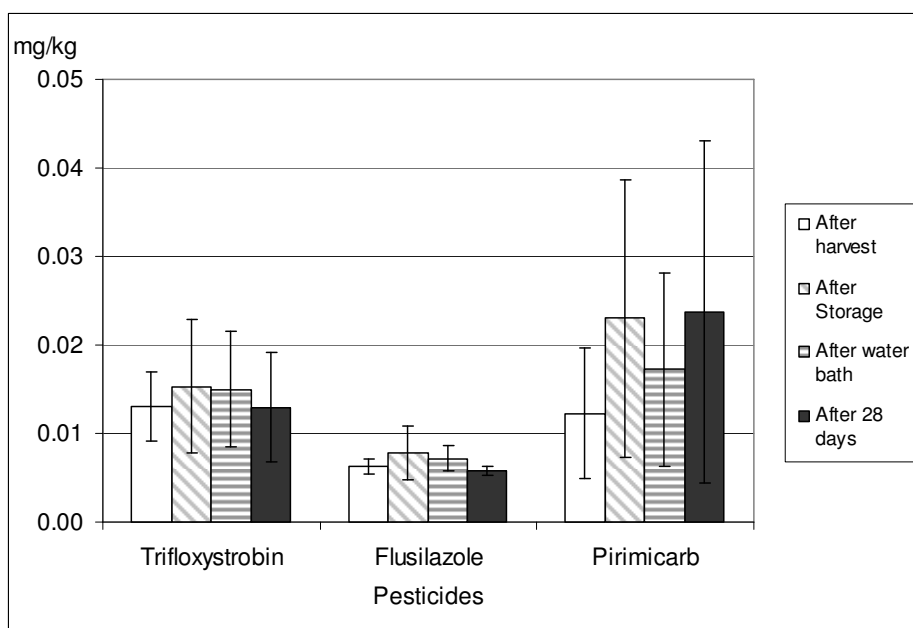


Figure 2. Residues of trifloxystrobin, flusilazole and pirimicarb in apples collected at the critical points along the artificial chain from producer to consumer.

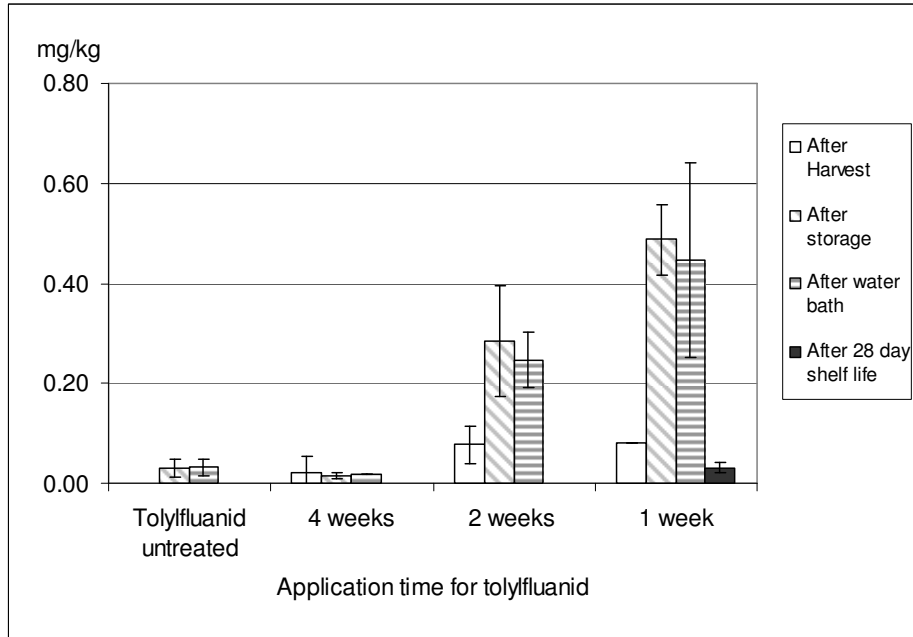


Figure 3. Residues of tolylfluaniid in apples, applied at different time and collected at the critical points along the artificial chain from producer to consumer.

Table 1. Application dates with the formulations and active substances used, apple group sprayed and number of days from spraying to harvest

Application no.	Application dates	Apple group sprayed	Formulations	Active substances	No. of days to harvest
1	26-05-2006	Group 1,2,3,4	Flint	Trifloxystrobin	112
2	08-06-2006	Group 1,2,3,4	Flint	Trifloxystrobin	99
3	17-06-2006	Group 1,2,3,4	Nustar Alanto	Flusilazole Thiacloprid	90
4	29-06-2006	Group 1,2,3,4	Nustar	Flusilazole	78
5	12-07-2006	Group 1,2,3,4	Flint	Trifloxystrobin	65
6	26-07-2006	Group 1,2,3,4	Flint Pirimor	Trifloxystrobin Pirimicarb	51
7	09-08-2006 31-08-2006 07-09-2006	Group 2 Group 3 Group 4	Euparene Euparene Euparene	Tolyfluanid Tolyfluanid Tolyfluanid	37 15 8

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Table 2. Schedule for sampling at critical point from the chain from orchard to consumer, group samples, number of samples and the analytical program performed

Critical point	Date	Apple group	Number of samples	Analytical profile
1) After harvest	15 September 2006	Group 1,2,3,4	4	Pesticides
2) After storage in CA Controlled Atmosphere	15 March 2007	Group 1,2,3,4	4	Pesticides
3) After grading/water bath	15 March 2007	Group 1,2,3,4	4	Pesticides
4) After 28 days of shelf-life	12 April 2006	Group 1,2,3,4	4	Pesticides and patulin

Table 3: Transitions for the validated pesticides and patulin.

Pesticide/Metabolite	Molecular formula	Ionisation, ESI +/-	Molecular ion	precursor	Transition 1			Transition 2		
					Product	Cone voltage (V)	Collision energy (eV)	Product	Cone voltage (V)	Collision energy (eV)
2,4-D	C ₈ H ₆ Cl ₂ O ₃	ESI-	[M-H]-	219	161	9	13	125	10	26
2-Naphtoxy acetic acid	C ₁₂ H ₁₀ O ₃	ESI-	[M-H]-	201	143	45	26	115	45	26
4-Chlorphenoxyacetic acid	C ₈ H ₇ ClO ₃	ESI-	[M-H]-	185	127	24	17	123	24	19
Acrinathrin	C ₂₆ H ₂₁ F ₆ NO ₅	ESI-	[M-H]-	540	513	45	11			
Acetamidrid	C ₁₀ H ₁₁ CIN ₄	ESI+	[M+H]+	223	126	27	20	90	27	35
Bentazone	C ₁₀ H ₁₂ N ₂ O ₃ S	ESI-	[M-H]-	239	132	38	29	196	38	21
Bupirimat	C ₁₃ H ₂₄ N ₄ O ₃ S	ESI+	[M+H]+	317	166	27	23	108	25	25
Buprofenzin	C ₁₆ H ₂₃ N ₃ OS	ESI+	[M+H]+	306	201	10	11	106	10	23
Clodinafoppropargyl	C ₁₇ H ₁₃ ClFNO ₄	ESI+	[M+H]+	350	266	38	17	91	38	20
Clomazone	C ₁₂ H ₁₄ CINO ₂	ESI+	[M+H]+	240	125	10	29	89	10	39
Dementon-S-methyl sulfoxid	C ₆ H ₁₅ O ₄ PS ₂	ESI+	[M+H]+	247	169	33	10	127	18	25
Demeton-S-methyl sulfon	C ₆ H ₁₅ O ₅ PS ₂	ESI+	[M+H]+	263	169	55	15	127	45	28
Dichlorprop (/Dichlorprop-P)	C ₉ H ₈ Cl ₂ O ₃	ESI-	[M-H]-	233	161	52	17	125	52	26
Dimethomorph	C ₂₁ H ₂₂ CINO ₄	ESI+	[M+H]+	388	301	45	20	165	23	30
DNOC	C ₇ H ₆ N ₂ O ₅	ESI-	[M-H]-	197	137	10	17	109	10	20
Epoxiconazole	C ₁₇ H ₁₃ ClFN ₃ O	ESI+	[M+H]+	330	121	45	23	91	45	41
Fenazaquin	C ₂₀ H ₂₂ N ₂ O	ESI+	[M+H]+	307	161	55	17	131	52	14
Fluoxastrobin	C ₂₁ H ₁₆ ClFN ₄ O ₅	ESI+	[M+H]+	459	427	45	17	188	45	37
Flusilazole	C ₁₆ H ₁₅ F ₂ N ₃ Si	ESI+	[M+H]+	316	165	51	20	247	20	17
Hexythiazox	C ₁₇ H ₂₁ CIN ₂ O ₂ S	ESI+	[M+H]+	353	228	45	11	168	45	27
Iodosulfuron-methyl	C ₁₄ H ₁₃ IN ₅ NaO ₆ S	ESI+	[M+H]+	530	163	21	13	390	21	14
Iprovalicarb	C ₁₈ H ₂₈ N ₂ O ₃	ESI+	[M+H]+	321	119	45	17	91	45	48
Isoproturon	C ₁₂ H ₁₈ N ₂ O	ESI+	[M+H]+	207	72	38	23	165	17	13
Malaoxon	C ₁₀ H ₁₉ O ₇ PS	ESI+	[M+H]+	315	127	48	10	99	33	21
MCPA	C ₉ H ₅ ClO ₃	ESI-	[M-H]-	199	141	55	11	105	55	26
Mecoprop (/Mecoprop-P)	C ₁₀ H ₁₁ ClO ₃	ESI-	[M-H]-	213	141	38	23	105	38	28
Metalaxyl -M	C ₁₅ H ₂₁ NO ₄	ESI+	[M+H]+	280	220	52	11	160	52	22
Methiocarb sulfoxide	C ₁₁ H ₁₅ NO ₃ S	ESI+	[M+H]+	242	185	33	10	122	33	30
Metsulfuron-methyl	C ₁₄ H ₁₅ N ₅ O ₆ S	ESI+	[M+H]+	382	167	52	17	135	52	32
Molinate	C ₉ H ₁₇ NOS	ESI+	[M+H]+	188	126	52	11	83	30	18
Monocrotophos	C ₇ H ₁₄ NO ₅ P	ESI+	[M+NH ₄]+	241	193	21	10	127	10	20
Monolinuron	C ₉ H ₁₁ CIN ₂ O ₂	ESI+	[M+H]+	215	126	55	17	99	52	30

Pesticide/Metabolite	Molecular formula	Ionisation, ESI +/-	Molecular ion	precursor	Transition 1			Transition 2		
					Product	Cone voltage (V)	Collision energy (eV)	Product	Cone voltage (V)	Collision energy (eV)
Omethoate	C ₅ H ₁₂ NO ₄ PS	ESI+	[M+H] ⁺	214	183	10	11	143	10	17
Pirimicarb	C ₁₁ H ₁₈ N ₄ O ₂	ESI+	[M+H] ⁺	239	72	25	16	182	25	14
Proquinazid	C ₁₄ H ₁₇ IN ₂ O ₂	ESI +	[M+H] ⁺	373	331	52	11	289	52	25
Prosulfuron	C ₁₅ H ₁₆ F ₃ N ₅ O ₄ S	ESI+	[M+H] ⁺	420	141	21	22	167		
Pyraclostrobin	C ₁₉ H ₁₈ ClN ₃ O ₄	ESI+	[M+H] ⁺	388	194	24	11	163	24	25
Pyridaphenthion	C ₁₄ H ₁₇ N ₂ O ₄ PS	ESI +	[M+H] ⁺	341	189	39	30	205	35	18
Pyridate	C ₁₉ H ₂₃ ClN ₂ O ₂ S	ESI+	[M+H] ⁺	379	207	45	17	104	45	30
Pyriproxyfen	C ₂₀ H ₁₉ NO ₃	ESI +	[M+H] ⁺	322	96	55	20	185	27	23
Spiroxamin	C ₁₈ H ₃₅ NO ₂	ESI +	[M+H] ⁺	298	144	51	20	100	35	30
Thiacloprid	C ₁₀ H ₉ ClN ₄ S	ESI+	[M+H] ⁺	255	128	30	20			
Thifensulfuron-methyl	C ₁₂ H ₁₃ N ₅ O ₆ S ₂	ESI-	[M-H] ⁻	386	139	31	25	220	31	5
Thiodicarb	C ₁₀ H ₁₈ N ₄ O ₄ S ₃	ESI +	[M+H] ⁺	355	88	27	15	108	27	15
Tolyfluanid	C ₁₀ H ₁₃ Cl ₂ FN ₂ O ₂ S ₂	ESI+	[M+H] ⁺	347	137	40	25			
Triasulfuron	C ₁₄ H ₁₆ ClN ₅ O ₅ S	ESI +	[M+H] ⁺	402	167	52	17	141	52	22
Trifloxystrobin	C ₂₀ H ₁₉ F ₃ N ₂ O ₄	ESI+	[M+H] ⁺	409	186	55	17	145	55	45
Triflumuron	C ₁₅ H ₁₀ ClF ₃ N ₂ O ₃	ESI +	[M+H] ⁺	359	156	21	25	139	20	30
Patulin	C ₇ H ₆ O ₄	ESI-	[M-H] ⁻	153	109	20	12	81	20	17

Table 4. Repeatability, reproducibility, recovery and limits of detection (LOD) at three spiking levels, for pesticides residues in apple.

Spiking concentration, mg/kg		0.02	0.04	0.2	LOQ, mg/kg
2,4-D	RSD _r , %		6%	8%	0.02
	RSD _R , %		7%	11%	
	Recovery, %		92%	88%	
Acetamiprid	RSD _r , %		23%	19%	0.06
	RSD _R , %		23%	19%	
	Recovery, %		101%	98%	
Acrinathrin	RSD _r , %	17%	7%	11%	0.03
	RSD _R , %	22%	26%	11%	
	Recovery, %	128%	105%	107%	
Bentazone	RSD _r , %	7%	9%	16%	0.01
	RSD _R , %	7%	17%	17%	
	Recovery, %	112%	125%	107%	
Bupirimate	RSD _r , %		14%	7%	0.03
	RSD _R , %		14%	31%	
	Recovery, %		94%	113%	
Buprofezin	RSD _r , %	21%	21%	14%	0.03
	RSD _R , %	26%	26%	14%	
	Recovery, %	89%	103%	117%	
Demeton-S- methyl Sulfoxid	RSD _r , %	29%	17%	18%	0.03
	RSD _R , %	29%	20%	18%	
	Recovery, %	88%	93%	107%	
Demeton-S-methyl Sulfon	RSD _r , %		4%	17%	0.03
	RSD _R , %		16%	17%	
	Recovery, %		87%	106%	
Dichloprop	RSD _r , %	11%	8%	18%	0.01
	RSD _R , %	14%	19%	18%	
	Recovery, %	85%	91%	90%	
Diethofencarb	RSD _r , %		19%	26%	0.05
	RSD _R , %		22%	26%	
	Recovery, %		90%	115%	
Fenazaquin	RSD _r , %	26%	18%	17%	0.02
	RSD _R , %	26%	21%	18%	
	Recovery, %	75%	93%	107%	
Flusilazole	RSD _r , %			8%	0.14
	RSD _R , %			11%	
	Recovery, %			99%	
Hexythiazox	RSD _r , %			20%	0.28
	RSD _R , %			20%	
	Recovery, %			117%	
Iprovalicarb	RSD _r , %	2%	17%	18%	0.02
	RSD _R , %	20%	18%	18%	
	Recovery, %	112%	92%	114%	
Isoproturon	RSD _r , %	19%	28%	16%	0.02
	RSD _R , %	19%	28%	16%	
	Recovery, %	104%	99%	110%	

Spiking concentration, mg/kg		0.02	0.04	0.2	LOQ, mg/kg
Malaaxon	RSD _r , %	14%	16%	12%	0.02
	RSD _R , %	14%	22%	12%	
	Recovery, %	102%	99%	107%	
MCPA	RSD _r , %	20%	21%	14%	0.03
	RSD _R , %	24%	21%	14%	
	Recovery, %	101%	70%	107%	
Mecoprop	RSD _r , %	8%	21%	19%	0.01
	RSD _R , %	14%	22%	19%	
	Recovery, %	94%	80%	89%	
Methalaxyl-M	RSD _r , %	21%	12%	3%	0.02
	RSD _R , %	21%	20%	8%	
	Recovery, %	99%	100%	107%	
Methiocarb sulfoxid	RSD _r , %	17%	4%	27%	0.02
	RSD _R , %	27%	19%	27%	
	Recovery, %	109%	99%	98%	
Metsulfuron-methyl	RSD _r , %		14%	17%	0.05
	RSD _R , %		24%	19%	
	Recovery, %		104%	121%	
Molinate	RSD _r , %		5%	4%	0.04
	RSD _R , %		17%	25%	
	Recovery, %		104%	92%	
Monolinuron	RSD _r , %	9%	23%	8%	0.04
	RSD _R , %	31%	23%	9%	
	Recovery, %	107%	96%	108%	
Omethoate	RSD _r , %	15%	18%	8%	0.02
	RSD _R , %	19%	35%	14%	
	Recovery, %	108%	106%	111%	
Pirimicarb	RSD _r , %	12%	7%	11%	0.02
	RSD _R , %	19%	23%	14%	
	Recovery, %	94%	89%	117%	
Pyraclostrobin	RSD _r , %	18%	24%	9%	0.02
	RSD _R , %	18%	24%	9%	
	Recovery, %	109%	105%	112%	
Pyriproxyfen	RSD _r , %	13%	6%	14%	0.02
	RSD _R , %	15%	20%	14%	
	Recovery, %	105%	130%	120%	
Spiroxamine	RSD _r , %	16%	16%	16%	0.02
	RSD _R , %	17%	25%	16%	
	Recovery, %	93%	90%	113%	
Thiodicarb	RSD _r , %	19%	24%	11%	0.01
	RSD _R , %	19%	28%	11%	
	Recovery, %	65%	96%	103%	
Triflumuron	RSD _r , %	28%	4%	20%	0.03
	RSD _R , %	30%	54%	20%	
	Recovery, %	78%	109%	113%	

Table 5. Mean recovery and standard deviation for patulin, trifloxystrobin, tolylfluanid, thiacloprid, flusilazole and pirimicarb in apples.

Patulin	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	86%	105%	101%	
Standard dev.	8%	22%	26%	0.04

Trifloxystrobin	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	84%	86%	89%	
Standard dev.	9%	9%	7%	0.01

Tolylfluanid	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	69%	87%	109%	
Standard dev.	19%	23%	20%	0.02

Thiacloprid	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	86%	92%	90%	
Standard dev.	13%	21%	11%	0.02

Flusilazole	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	91%	81%	86%	
Standard dev.	13%	6%	14%	0.02

Pirimicarb	Spiking level, mg/kg			LOQ mg/kg
	0.02	0.04	0.2	
Mean	96%	97%	97%	
Standard dev.	4%	15%	4%	0.005

Table 6. Patulin content in apples treated with pesticides at different times before harvest. Both the non-inoculated and the samples inoculated with *P. expansum* were investigated after 28 days of shelf-life

Pesticide treatment	Patulin content (mg/kg) in non inoculated samples	Patulin content in core inoculated samples (mg/kg)	Patulin content in surface inoculated samples (mg/kg)
Untreated	< 0.02 (-)	15.4 (+)	4.1 (+)
Treated with pesticides 4 weeks before harvest	< 0.02. (-)	not determined	not determined
Treated with pesticides 2 weeks before harvest	< 0.02. (-)	8.7 (+)	4.3 (+)
Treated with pesticides 1 week before harvest	< 0.02. (-)	15.2 (+)	2.7 (+)