

Original Research

**DIRECT INFUSION MS AS A PROMISING TOOL FOR PESTICIDES
QUALITATIVE SCREENING IN APPLES**

DOI: 10.5937/EcoTER26297A

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Abstract

Frequent pesticide application, along with their health risk keep the need for continuous pesticides residue monitoring in food. Nowadays multiresidue pesticides analysis in complex matrices is mostly quantitative chromatography-based analysis. Methods that are primarily designed for qualitative analysis and exclude the usage of chromatography much less can be found in literature. Primary goal of this investigation was to launch a non-chromatography based direct infusion ESI/MS method designed for fast qualitative pesticides screening in fruit. The method was intended to be shorter and simpler than usual methods. As such, the method can be used in early stages in pesticides residues evaluation process, and the obtained results can decide whether to proceed evaluation with some quantitative method or do not.

Keywords: mass spectrometry, sample preparation, fungicides.

INTRODUCTION

The main contribution of vitamins and nutrients to the human diet originates from fruits and vegetables, among which apples are the most consumed. Around 80 million metric tons of this fruit is predicted to be globally produced in 2022/23, of which is about half a million to 13 million tons in EU only [1]. Apple production is highly dependent on pesticides since the usage of these chemicals ensures satisfactory crop yields. Frequent pesticide application, along with their health risk keep the need for continuous pesticides residue monitoring in food.

Nowadays multiresidue pesticides analysis in complex matrices is mostly quantitative chromatography-based analysis. Methods that are primarily designed for qualitative analysis and exclude the usage of chromatography much less can be found in literature.

Primary goal of this investigation was to launch a non-chromatography based direct infusion ESI/MS method designed for fast qualitative pesticides screening in fruit. The method was intended to be shorter and simpler than usually applied mostly quantitative

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methods. As such, the method can be used in early stages in pesticides residues evaluation process, and the obtained results can decide whether to proceed evaluation with some quantitative method or do not.

Due to its cuticle structure and composition, apple peel was targeted for analysis. Peel is a natural barrier, thus can retain most of the applied pesticides. Peel retention capacity of several fruits (apple, grape, pear) was confirmed by some studies [2–4] that non-systemic pesticides were better localized than the systemic, since the latter express deeper penetration pattern [5,6].

The method was developed for five pesticides mostly used in apple production in Serbia - pyrimethanil, cyprodinil (fungicides, anilino-pyrimidines), boscalid (fungicide, pyridine-carboxamides), trifloxystrobin (fungicide, Strobilurin) and bifenthrin (insecticide, pyrethroids).

MATERIALS AND METHODS

Chemicals

High purity standards of pyrimethanil and cyprodinil were purchased from AccuStandard®, USA. Formic acid (98%) and high purity standards of boscalid, trifloxystrobin and bifenthrin were purchased from Sigma-Aldrich®, Germany. HPLC grade methanol was produced by J.T. Baker, USA; ammonium-formate (98%) and HPLC grade hexane, deionized water and ethanol were produced by Carlo Erba, Italy.

For high purity standards weighing procedures analytical balance Sartorius BP110S (Germany) was used.

In sample preparation procedure following appliances were used: peeler and slicer (China), balance (acc. $\pm$ 0.01 g) KB 2000-2N KERN (Germany), kitchen blender 0.9L BL142A TEFAL (France), centrifuge Jouan C4i Thermo Scientific (USA), evaporator TurboVap® LV Zymark (USA), hotplate Harry Gestigkeit GmbH (Germany), syringe microfilter Nylon Hydrophilic 0.22 μ m, Membrane Solutions (USA) and 500 μ L glass syringe, Unimetrics (USA). Apples of Golden Delicious variety were purchased in a local supermarket.

Instruments and instrumental configuration for screening analysis

Surveyor LC pump (Thermo Finnigan, USA) and LCQ Advantage mass spectrometer (Thermo Finnigan, USA) with 3D ion trap analyzer, were used. Data was acquired and analyzed by Xcalibur™ software, version 1.4 SR1.

LC pump and mass spectrometer were coupled via PEEK tubing system interconnected with LC union Tee (syringe - LC union Tee branch (30 cm) and LC union Tee - ESI source branch (10 cm)) (Figure 1). The configuration provides simultaneous inflow of mobile phase from the LC pump and sample from the syringe pump to the mass spectrometer. The mobile phase inflow (0.30 % FA + 0.01 % AMF water solution) can be delivered by LC pump or any other pump. The aim is to provide and maintain constant environment in the source during sequential analysis; optionally, it gives an opportunity to adjust parameters such as pH, salt presence etc.

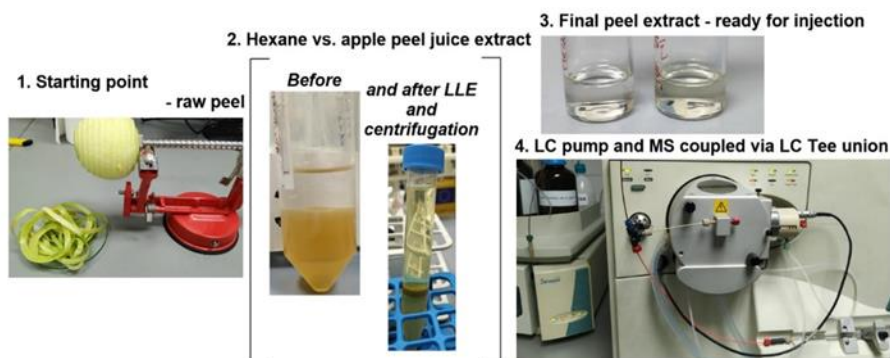


Figure 1 An insight into screening analysis

Sample preparation

Procedural blanks

Ten-gram peel portion was mixed with water (20 mL) and blended. The obtained mash was centrifugated (10 min/3500 rpm); filtrate (15 mL) was liquid-liquid extracted with hexane (10 mL); hexane extract (6 mL) was separated from aqueous phase by centrifugation (3 min/3500 rpm), evaporated to dryness, redissolved in 1.50 mL 0.05% AMF in methanol, filtered through 0.22 μm syringe filter and analyzed.

Procedural standards

Ten-gram peel portion was fortified with five-pesticides mixture as follows: calculated volume of pesticides stock solution was applied to the peel surface with a pipette, taking care all the solution was uniformly distributed. Upon solvent evaporation, peel was capped and refrigerated for 30 minutes. Fortified peel was then treated according to the procedural blanks' preparation procedure. Fortification levels were 0.05; 0.10; 0.20; 0.50 and 0.75 mg kg^{-1} of peel.

Screening analysis steps

Extracts were injected into the source in purge syringe mode (25.00 $\mu\text{L min}^{-1}$); simultaneous mobile phase inflow rate was 50.00 $\mu\text{L min}^{-1}$. Screening analysis was performed in two steps. First step was the MS^1 analysis (full scan mode, scan range m/z 150-600). The second step was the subsequent MS^2 analysis (full scan mode) of selected pesticide-specific parent ions (Table 1). Total runtime was 12 min (2 min for MS^1 analysis and 2 min for MS^2 analysis of each pesticide).

Table 1 Analytically significant ions (m/z) of investigated pesticides

Pesticide	Pyrimethanil	Cyprodinil	Boscalid	Trifloxystrobin	Bifenthrin
Molecular weight, gmol^{-1}	199.25	225.29	343.2	408.4	422.9
m/z of $[\text{M}+\text{H}]^+$ (monoisotopic)	200.12	226.13	343.04	409.14	423.13
Base ion observed in the MS^1 spectra (parent ion for MS^2), m/z	200.4	226.4	342.9	408.7	439.6
Daughter ions in the MS^2 spectra, m/z (descending abundance)	107.1 183.4 159.2	210.2 185.1 144.1 159.0 108.1	307.3 140.0	186.1 206.1	181.1 166.1
Normalized collision energy, %	50 (55) ^a	55	35 (45) ^a	30 (40) ^a	23 (50) ^a

^a At this value parent ions of pesticides are completely converted into daughter ions.

RESULTS AND DISCUSSION

Method development

Two possible outcomes of the screening are the confirmation of pesticides presence or absence. In first case, pesticides amount in apples can be assessed by methods approved by national or EU/USA regulations. In latter case, the analysis finishes at that point *i.e.*, there is no need to conduct any further analysis. The method itself excludes complex sample preparation or expensive chemicals consummation (sorbent packages, salts etc.), thus offering both time- and cost-effectiveness when analyzing large number of samples, which might not be the case with certified quantitative methods.

First step of the sample preparation was peel blending assisted with the addition of water. Since water is a non-aggressive medium, extraction can be performed in any type of blender; also, highly abundant waxes are not extracted. The second step was the liquid-liquid extraction of filtered water extract with hexane. Hexane does not extract salts from the initial extract, which is important, since larger quantity of non-volatile salts injected directly into the source in a short period of time can form deposits and cause clogging problems.

Dry residue amount in peel extracts was determined in a separate experiment. The amount originating from 50 g of peel after evaporation for 24 h at room temperature was around 1 mg (1.15 ± 0.12 mg).

Heating (30 min at 50 °C) decreased the amount to 0.2 mg and further heating (30 min at 100 °C) resulted in no measurable amounts, suggesting the deposition in the source is improbable, since the screening starts with 10 g of peel and the lowest capillary temperature in source is usually 200 °C, which surely provides complete evaporation of any dry residue.

Complete prevention of waxes occurrence in hexane extract is impossible since the final water extract is cloudy (Figure 1). Our previous study [7] showed the final sample preparation step - hexane to methanol exchange by evaporation - provides the complete removal of extracted waxes. The step also provides analyte concentration and, in this case, more appropriate analyte carrier when operating with ESI/MS systems.

Qualitative analysis

Ions originated from peel extracts are mainly observed at m/z values higher than 290 (Figure 2). Background spectrum (mobile phase only) and blank apple extracts spectra differ in 5 to 6 additional ions of higher abundance.

MS^2 analysis was performed on all fortified and non-fortified samples. Detected pesticides pseudo-molecular ions were fragmented at both lower and higher normalized collision energy; for MS^2 fragments quantification higher energy was used (the value is given in parenthesis in Table 1).

Pesticides' fragmentation patterns in the neat solution and in spiked procedural blank ($5.00 \mu\text{g mL}^{-1}$) (spectrum can be found in [8]) are similar *i.e.*, there are no new-formed fragments. However, in spiked extract smaller number of pyrimethanil and cyprodinil fragments was observed; most characteristic fragments of each pesticide (first two fragments, Table 1) are among them. Boscalid, trifloxystrobin and bifenthrin fragmentation patterns do not differ.

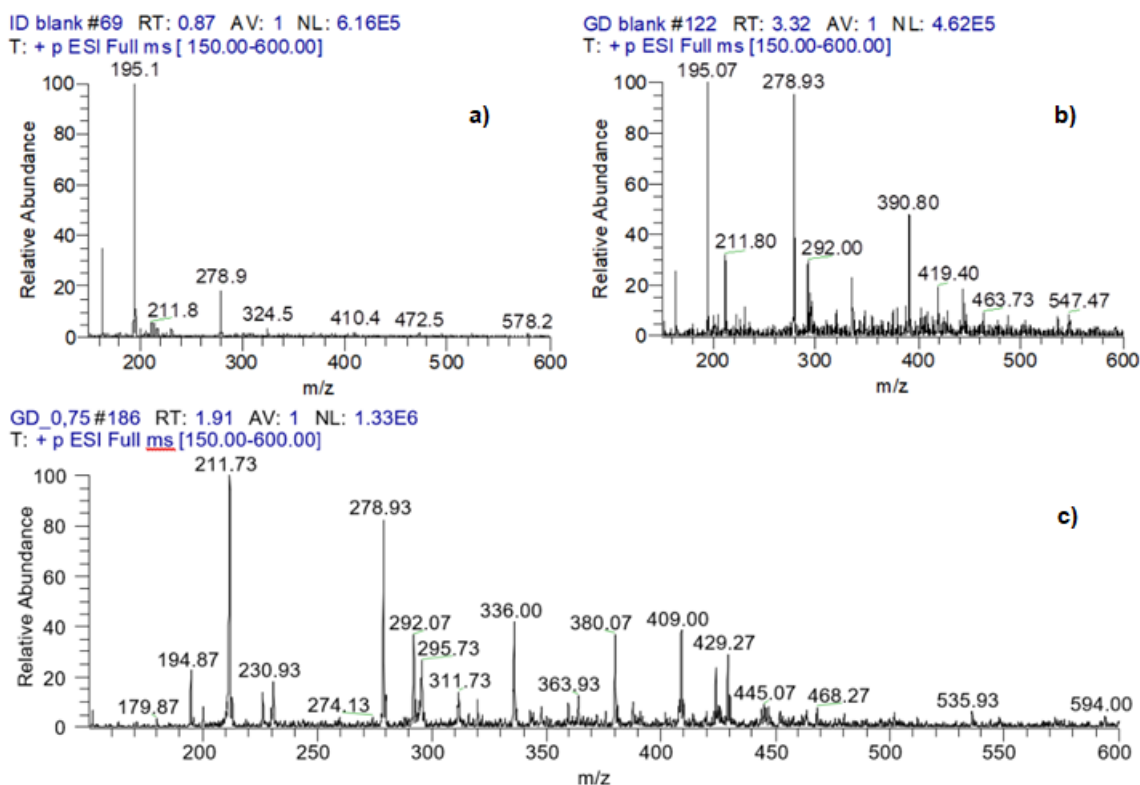


Figure 2 Full scan MS^1 background spectra (mobile phase only) (a); full scan MS^1 spectra of non-fortified (b) and fortified Golden Delicious peel extract (0.75 mg kg^{-1}) (c)

Comparison of pesticides fragmentation pattern in procedural standard (0.050 mg kg^{-1} , Figure 3) to the pattern in the non-procedural analogue (procedural blank spiked at $0.25 \mu\text{g mL}^{-1}$, spectrum can be found in [8]) shows the following:

- Fragmentation of pyrimethanil (m/z 200) results in m/z 183, 173, 131 and 85 (Figure 3a) of which m/z 183 and perhaps m/z 173 can be related to the pesticide; m/z 107 is not observed. At higher collision energy (CID = 55) m/z 183 becomes dominant. In 0.75 mg kg^{-1} fortified peel (spectrum can be found in [8]) three pyrimethanil characteristic fragments are observed (m/z 107, 125 and 183) which clearly confirms the pesticide presence. At higher concentrations, the most abundant pyrimethanil fragment is m/z 107 (spectrum can be found in [8]). However, at lower concentrations m/z 183 becomes more reliable for pyrimethanil identification.
- Presence of cyprodinil can be reliably confirmed by observing at least four characteristic fragments i.e., m/z 210, 159, 133 and 108 (Figure 3b).
- Presence of boscalid, trifloxystrobin and bifenthrin can be also reliably confirmed by observing m/z 307 and 140 (Figure 3c), m/z 186 and 206 (Figure 3d) and m/z 181 and 166 (Figure 3e).

Qualification of boscalid, trifloxystrobin and bifenthrin in apple extracts is reliable since the fragmentation pattern is well preserved and mostly unchanged at different pesticides concentration in samples. Fragmentation patterns of cyprodinil and pyrimethanil are altered in terms of lower diversity of the fragments. This is more pronounced for pyrimethanil,

particularly at lower concentrations, and might cause the identification of this pesticide to be slightly challenging.

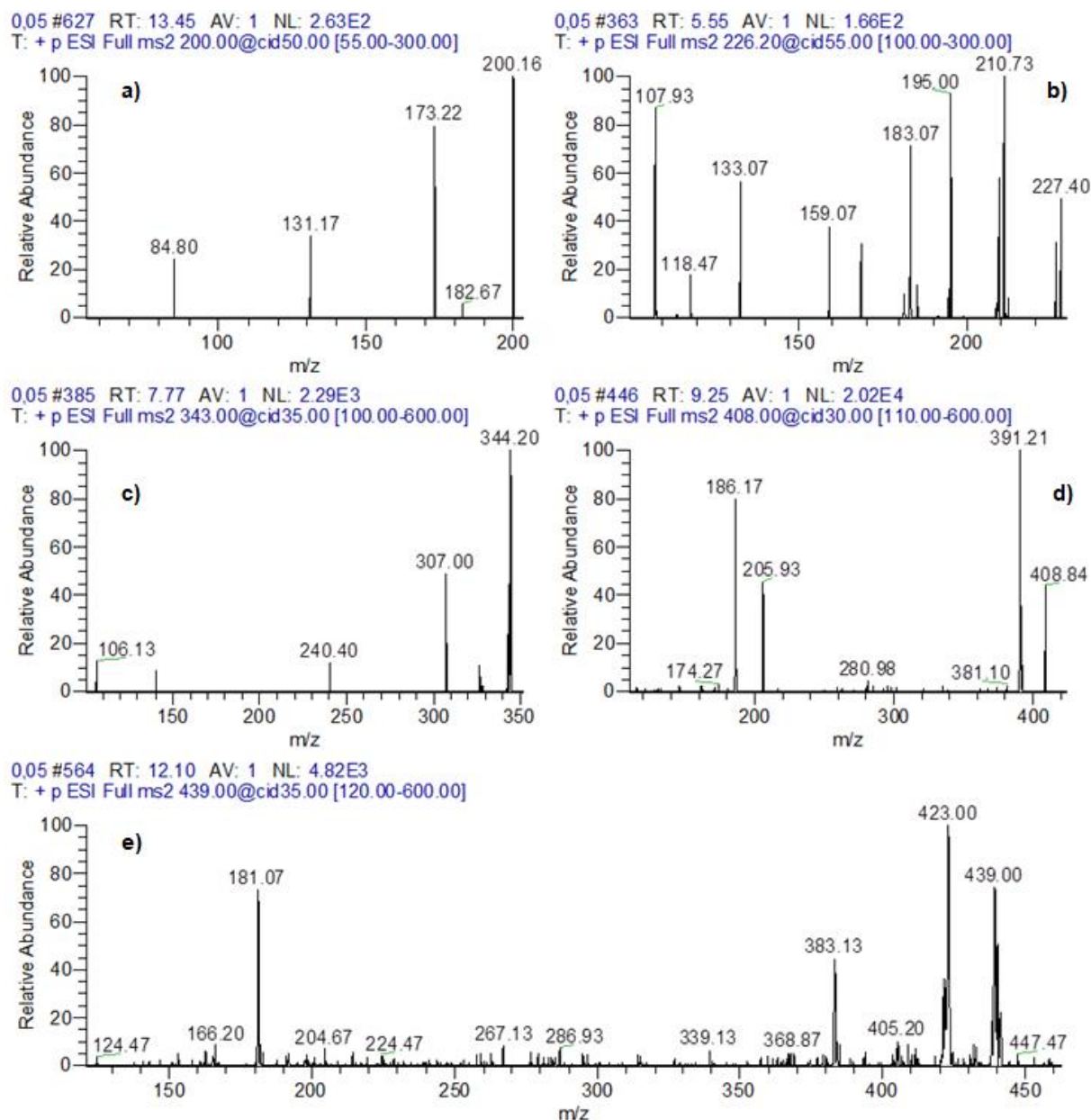


Figure 3 Direct injection full scan MS² spectra after the fragmentation of protonated molecular ion of: a) pyrimethanil (m/z 200.4); b) cyprodinil (m/z 226.4); c) boscalid (m/z 343.0); d) trifloxystrobin (m/z 408.7); and e) bifenthrin (m/z 439.7) in extract of fortified Golden Delicious peel (0,050 mgkg⁻¹)

CONCLUSION

The developed method involved direct infusion ESI/MS analysis on 3D ion trap mass spectrometer. ESI behavior and fragmentation pattern of selected pesticides were first evaluated by MS¹ and MS² analysis of pesticides neat solutions, then same type of analysis was performed on a series of fortified Golden Delicious peel samples to assess method's qualification parameters.

Qualification criteria were the observation of MS² pesticide-characteristic fragments in samples spectra. Following the criteria, pesticides were detected in the lowest fortified sample (0.05 mgkg⁻¹ of peel or approx. 0.005 mgkg⁻¹ of fruit), meaning the method can qualify pesticides below MRL levels. Qualification of one of five investigated pesticides can be slightly challenging since the fragmentation in extracts leads to a smaller number of pesticide fragments when compared to that in the neat solution.

The developed method can be considered cost-effective and fast since the sample preparation itself does not include any expensive reagents and the chromatography was omitted.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia for financial support according to the contract with registration No. 451-03-33/2026-03/200124 and 451-03-34/2026-03/200383.

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