

Pesticide residues in commercially available oranges and evaluation of potential washing methods

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Abstract. Pesticides, which are widely used in agriculture to increase production or protect crop, are known to be potentially dangerous to consumers. Therefore oranges from local supermarket were analysed by the LC–ESI–MS method to determine the presence of pesticides. Two pesticides – thiabendazole and imazalil – were found in the oranges. Both are post-harvest pesticides. Analyses showed that these pesticides are located in and on the peel of oranges. Five different washing methods – washing with cold water, with hot water, with soap, with dishwashing soap, and treating with ultrasound – were evaluated for their efficiency in removing pesticide residues.

Key words: pesticide residue analysis, LC–MS, ESI, electrospray ionization.

INTRODUCTION

Pesticides are widely used in agriculture to fight weeds, moulds, and pests for increasing productivity. Besides this positive effect pesticides pose health-risk to consumers [1]. Therefore concentration of pesticide residues in many products, including fruit and vegetables, must be monitored and regulations such as SANCO/825/00 [2] have been developed. Among other analytical methods liquid chromatography (LC)–mass spectrometry (MS) with electrospray ionization (ESI) has been widely used to determine pesticide residues. To achieve high selectivity MS detection in multiple reaction monitoring mode is needed [3]. For sample preparation the novel QuEChERS (quick, easy, cheap, effective, rugged,

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safe) method [4] was used in this work. This method, based on the removal of co-extracted compounds with solid phase, is known to be very effective and economical.

Pesticides may be introduced to fruit and vegetables during different phases of production. Some pesticides are used before blooming, some while fruit are growing, and others after harvesting. Therefore the location of pesticides in the fruit may be different as well [5, 6]. Post-harvest pesticides are the major source of synthetic pesticides in food.

In order to protect ourselves from potential danger of pesticides fruit and vegetables are washed and peeled before consumption. As a novel method, washing in the presence of ultrasonic waves, has been introduced lately. Washing in ultrasonic baths is stated to remove agricultural residues and to kill bacteria [7].

The aim of this work was to find out the distribution of pesticide residues in commercially available oranges. Also five different washing methods were studied in order to find out the most effective one for removing pesticide residues.

EXPERIMENTAL

Reagents

Solvents, acetonitrile (J. T. Baker, Deventer, The Netherlands), and methanol (J. T. Baker) were of sufficient purity. The water used was purified with Millipore Simplicity 185 (MILLIPORE GmbH, Molsheim, France). Salts, magnesium sulphate, and sodium acetate were from Reakhim (Leningrad, Soviet Union). Before usage the magnesium sulphate was baked for 5 h at 500°C in a muffle furnace to remove possible phthalate impurities. Glacial acetic acid (Lach-Ner, Neratovice, Czech Republic) was used to improve the stability of base-sensitive pesticide residues in the final extract of the QuEChERS method.

Pesticide (aldicarb sulphoxide, aldicarb sulphone, demeton-S-methyl sulphoxide, carbendazim, methomyl, thiabendazole, methiocarb sulphoxide, methiocarb sulphone, aldicarb, imazalil, thiodicarb, phorate sulphoxide, phorate sulphone, methiocarb) standard substances were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of 1000 mg/kg in the appropriate solvent were prepared. The stock solution for carbendazim was 80 mg/kg because of its poor solubility. The working standard solution contained 14 pesticide residues at 40 mg/kg. For spiking appropriate dilutions were made. Primary Secondary Amine (PSA) (Supelco, Bellefonte, USA) was used in sample preparation.

Formic acid (Riedel-de-Haën) and ammonium acetate (Fluka Chemie AG, Buchs, Germany) were used for preparing eluents for LC.

Sample preparation

Fifteen gram of the homogenized sample was placed into a 50 mL polyethylene centrifuge tube. Then 15 mL of 1% acetic acid in acetonitrile (v/v), 6 g of anhydrous magnesium sulphate, and 1.5 g of anhydrous sodium acetate were added and the tube was vigorously shaken by hand for 1 min. It was necessary to ensure that the solvent interacts well with the entire sample and that the crystalline agglomerates are broken down sufficiently. The tube was centrifuged at 3000 rpm (900 g) for 1 min. The upper layer, the extract, was introduced into a glass centrifuge tube, which contained 50 mg of PSA and 150 mg of anhydrous magnesium sulphate per 1 mL of extract. The tube was sealed and shaken vigorously for 30 s. The tube was centrifuged at 3000 rpm for 1 min. The pre-concentration factor was 1.

Analytical instrument

The extracts were analysed with an Agilent Series 1100 LC/MSD Trap XCT (Santa-Clara, USA) instrument using electrospray ionization in the positive ion mode. The LC instrument was equipped with a binary pump, autosampler, thermostatted column compartment, and diode array detector. The mass spectrometer uses a quadrupole ion trap mass analyser. For instrument control Agilent ChemStation for LC Rev. A. 10.02 and MSD Trap Control version 5.2 were used. Data analyses were performed by Quant Analysis for LC/MSD Trap 1.6 and Data Analysis for LC/MSD Trap 3.2.

LC–MS–MS analyses

Chromatographic separation was carried out on 250 mm long Zorbax Eclipse XDB-C18 column with the internal diameter of 4.6 mm, particle size 5 μm . Also an Eclipse XDB-C18 12.5 mm long precolumn with the internal diameter of 4.6 mm and particle size 5 μm was used. An autosampler was used to inject 10 μL of probe solution. Gradient elution with methanol and buffer solution (pH = 2.8) was used. The buffer solution, as well as methanol, contained 1 mM ammonium acetate and 0.1% formic acid. The linear gradient started at 20% methanol and was raised to 100% within 15 min, then the column was eluted 17 min with methanol, and the methanol content was lowered to 20% in 3 min. The flow rate of the eluent was 0.8 mL/min.

During the analysis nitrogen was used as the nebulizing gas (40.0 psi = 276 kPa) and drying gas (10 L/min, 350°C). All other ESI and MS parameters were optimized individually for each pesticide residue. Table 1 lists the parameters for thiabendazole and imazalil – the two pesticides actually detected in this work. The mass spectrometer was operated in the multiple reactions monitoring mode (MRM).

Table 1. ESI and MS parameters for MS² detection of thiabendazole and imazalil

Parameter	Thiabendazole	Imazalil
Retention time, min	9.5	14.0
Capillary, V	-1175.1	-1267.21
Skimmer, V	30	35.5
Cap exit, V	69.18	73
Oct 1 DC, V	12.8	11
Oct 2 DC, V	1.13	1.12
Trap drive	31.57	34.51
Oct RF	80	90
Lens 1, V	-3	-3.9
Lens 2, V	-52.37	-50
Parent ion <i>m/z</i>	202	297
Fragment ion <i>m/z</i>	175	201
Fragmentation amplitude	0.46	0.56

Calibration

Calibration solutions were prepared daily from 40 mg/kg standard solution by consequent dilutions. For calibration three solutions with concentrations 1.00, 0.50, and 0.10 mg/kg were used.

Washing

Washing with cold water, hot water, soap, and dishwashing soap was carried out during 30 s in order to mimic real washing processes at home or in restaurants. The temperature of cold water was approximately 15°C. The temperature of hot water was between 60 and 70°C. Also household soap and dishwashing soap (Fairy) were used. Washing in the ultrasonic bath was carried out during 10 min at room temperature.

RESULTS AND DISCUSSION

Distribution of pesticides

In order to determine the location of pesticides in oranges four oranges were analysed. Two of them were homogenized as a whole (peel and pulp together). For the other two oranges peel and pulp were homogenized separately. With each of the homogenates sample preparation was carried out. The obtained extracts were injected into the analytical column.

The results are shown in Table 2. Out of the 14 pesticides analysed two – thiabendazole and imazalil (Fig. 1) – were found in oranges. The maximum residue limit for those two pesticides in oranges is 5 mg/kg [8]. So the level of pesticide residues in oranges was acceptable. Repeatability of sample preparation expressed as RSD was determined from a separate experiment: 6% for thiabendazole and 12% for imazalil.

Table 2. Distribution of pesticides

	Thiabendazole, mg/kg	Imazalil, mg/kg
Peel	0.29	0.64
Pulp	0.00	0.04
Whole fruit	0.07	0.20

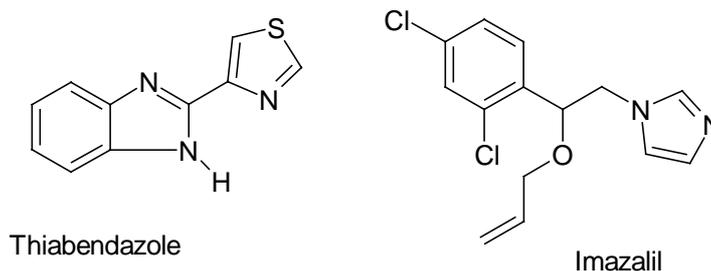


Fig. 1. Structures of thiabendazole and imazalil.

As the results show, both thiabendazole and imazalil were found in high concentrations in the peel of oranges. In the pulp the concentration of thiabendazole was below detection limit and that of imazalil was only 0.04 mg/kg.

Thiabendazole and imazalil are both mainly used as post-harvest pesticides [9]. This means that they should be located on the peel of oranges, which was also confirmed by our results. At the same time a small amount of imazalil was found in the pulp as well. This fact can be explained by diffusion of imazalil through the peel of oranges. The tendency of diffusion can be explained by high polarity of imazalil. The logarithm of octanol–water distribution coefficient for thiabendazole and imazalil is 2.47 and 3.82, respectively. The $\log P$ values were calculated using the SPARC on-line property estimation system of the University of Georgia (UGA), Department of Chemistry, Athens, GA, USA, 2007 (available from <http://ibmlc2.chem.uga.edu/sparc/>). The inside of oranges is more polar because of the high content of water. This explains the tendency of polar imazalil to diffuse through the orange peel.

Efficiency of washing

Five different methods of washing were applied to five oranges. After treatment the oranges were cut into four equal quarters and the peels of opposite quarters were combined and homogenized. The homogenized peel of both halves was analysed and the mean results are presented in Table 3. Analyses were carried out using only peel in order to improve detection and quantitation.

Table 3. Effects of washing oranges on thiabendazole and imazalil. Concentrations are presented in mg per 1 kg of orange peel

Treatment	Thiabendazole, mg/kg	Imazalil, mg/kg
Without washing	0.28	0.93
Cold water	0.24	0.58
Hot water	0.00	0.56
Soap	0.13	0.92
Dishwashing soap	0.00	0.67
Ultrasonic bath	0.13	0.44

As can be seen for thiabendazole all washing methods decrease the concentration. Washing with cold water is the least effective method of washing. Washing with soap and in ultrasonic bath decrease the concentration twice. Hot water and dishwashing soap are able to totally remove thiabendazole residues from the orange peel.

For imazalil the situation is not so straightforward. None of the methods used was able to remove imazalil totally. Washing with cold and hot water decreased the concentration by about one third, but using soap was ineffective. From all the used methods, washing in ultrasonic bath was the most effective in removing imazalil.

Diffusion of imazalil

The location of imazalil in oranges as well as washing results support the hypothesis of imazalil diffusion. If imazalil is located inside the peel rather than on the peel it is clear that the usual washing methods that can remove compounds from the surface are not able to remove imazalil effectively. As washing with cold and hot water still reduced the concentration of imazalil, it can be concluded that not all imazalil used had diffused into peel. The effectiveness of ultrasonic treatment in removing residues of imazalil may result from two effects: ultrasound facilitates diffusion of imazalil out of the peel pores or imazalil decomposes by treatment with ultrasound.

It has been reported in the literature [10] that in apples imazalil is found not only on the peel but also in the pulp. This fact supports the conclusion of imazalil's high diffusion rate.

These findings show that even if the pesticides are used post-harvest, they may diffuse through the peel of fruit and are also found in the pulp.

CONCLUSIONS

The aim of this work was to evaluate the distribution of pesticides in oranges and find out the most effective washing method for removing pesticides.

Residues of two pesticides – thiabendazole and imazalil – were present in oranges. The concentration of the pesticide residues was found to be higher in the peel, but a small amount of imazalil was present in the pulp as well. Washing with hot water and with dishwashing soap removed residues of thiabendazole, but none of the used methods could completely remove imazalil. The reason for this is the diffusion of polar imazalil into and through the peel of oranges. So consumers cannot be sure of avoiding post-harvest pesticides just by washing. Some residues of pesticides will be present even after removing the peel.

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Apelsinide pestitsiidijääkide analüüs ja puuviljade pesemisviiside efektiivsuse hindamine

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Pestitsiide kasutatakse laialdaselt puu- ja köögiviljade kaitseks umbrohu, kahjurite, hallituse ning mädanike vastu. Toksilisuse tõttu on pestitsiidijääkide sisaldused toiduainetes rangelt normeeritud. Pestitsiidijääkide analüüs nõuab proovide ettevalmistuseks ja määramiseks efektiivseid meetodeid.

Artiklis on pestitsiidijääkide määramiseks kasutatud uudset QuEChERS-proovide (*quick, easy, cheap, effective, rugged, safe*) ettevalmistamise meetodit, mis on pestitsiidijääkide analüüsil kujunemas mitmete riikide standardmeetodiks. Pestitsiidijääkide sisaldus prooviekstraktis on määratud LC-ESI-MS-meetodil (elektropihustusionisatsiooniga vedelikkromatograafia-massispektrometria). Kasutatud meetodika võimaldab määrata 14 pestitsiidi jääke. On uuritud ühest Tartu kauplusest ostetud apelsine. On tuvastatud kahe pestitsiidi (tiabendasool ja imasaliil) esinemine proovides koguses, mis ei ületa nende kehtestatud piirnorme. Kuna on teada, et tiabendasooli ja imasaliili kasutatakse viljade korjajärgseks töötlemiseks, siis on uuritud nende jaotumist apelsinikoore ja viljaliha vahel. On leitud, et pestitsiidijäägid ongi põhiliselt apelsinikoores või selle pinnal (tiabendasooli 0,29 mg/kg, imasaliili 0,64 mg/kg). Väikeses kontsentratsioonis on imasaliili leitud ka viljalihast – 0,04 mg/kg.

On võrreldud meetodeid, mida kasutatakse puuviljade pesemiseks kodus või tootlustusasutuses: pesemine külma ja kuuma veega, seebiga, nõudepesuvahendiga ning ultrahelivannis. Tiabendasooli korral on leitud, et pesemine kuuma vee või nõudepesuvahendiga kõrvaldab pestitsiidijäägid täielikult. Imasaliilijääke pole ükski pesemismeetod võimeline täielikult kõrvaldama. Kõige efektiivsemaks võib pidada töötlust ultrahelivannis. Pesemisekperimentide tulemused näitavad, et kui tiabendasoolijäägid paiknevad apelsinikoore pinnakihis, siis imasaliil on difundeerunud koore sisemusse. Imasaliili difundeerumise kohta läbi õunakoore on olemas ka kirjanduslikke andmeid. Tiabendasooli ja imasaliili erineva difundeerumise põhjuseks tuleb pidada nende ühendite erinevat polaarsust.