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Research Article

Determination and Extraction of Acetamiprid Residues in Fruits and Vegetables

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Abstract

Vegetables (chilies, tomato, cauliflower and cucumber) and fruits (mango and apple) samples were spiked with known quantity (0.50 mg kg-1) of acetamiprid reference standard for testing the retrieval percentage of acetamiprid residue in those vegetables and fruits. The efficiency of different extracting (ethyl acetate and dichloromethane + acetone 8:2) and eluting (ethyl acetate and dichloromethane + acetone 8:2) solvents and adsorbents (activated charcoal and florisil) for cleanup purpose was calculated using HPLC. Amongst the extracting solvents ethyl-acetate was observed an effective extracting solvent alone which produced maximum 90-96% recovery for acetamiprid residues while among the eluting solvents a combination of dichloromethane and acetone (ratio 8:2) produced superior recoveries i.e. 87-95%. Similarly, between the adsorbents used for cleanup purpose activated charcoal and florisil in tandem (first from charcoal and then through florisil) yielded recoveries 82-90 % whereas adsorbents used alone in form of activated florisil and charcoal recovered only 70 to 78 % and 71 to 73% acetamiprid residues, respectively in all vegetables and fruits.

1. Introduction

Food is the basic requirement of life and is a mean for Both vegetables and fruits are necessary elements for balanced diet because they protect human from cancer and other diseases owing to their richness in vitamins, minerals and fibers. The tool for their protection and production is synthetic chemicals. Pesticides have potentially adverse effects on fruits, vegetables, crops and human health (Kumari, Madna, & Kathpal, 2006; Kumari et al., 1996). The estimation of pesticide residues in horticultural crops, i.e. fruits and vegetables is necessary to appraise the potential threat to end users. The substantial but injudicious use of pesticides by farmers pollutes the soil, air and water environments; several types of crops and eventually the human beings (Hamilton & Crossley, 2004). Consequently the research about pesticide residues in fruits and vegetables is imperative owing to its direct relationship with health of masses, environment and new era of global trade (Wilson and Outsuki, 2001; Wilson & Outsuki, 2002; FAO-IDB, 2003; Mukherjee et al., 2007).

Acetamiprid (E) – N^1-[(6-chloro-3-pyridinyl) methyl] – N^2- cyano –N1-methyl acetamidine is a broad spectrum neonicotionoid insecticide with outstanding systemic translaminar activity. It directly affects the CNS (central nervous system) of the insect through disrupting the acetylcholine receptor in the synapses. It is used for the control of hemiptera, aphids etc. through soil and foliar application on a number of crops (cotton, sugar beet, vegetables, fruits and tea). It is readily soluble in acetone, dichloromethane and methanol; stable in acidic to neutral aqueous solution but totally unstable under alkaline conditions.

Acetamiprid in human body is quickly absorbed (>96% after 24h) and almost completely excreted (>90% after 96h), mainly via urine. In Plants it is degraded or formed five metabolites. (Philip et al., 2003; Tomlin, 2006). Ferrer et al. (2005) studied acetamiprid resides in fruits (apple, orange, lemon and melon) and vegetables (pepper, broccoli and tomato) using ethyl acetate as extraction solvent by liquid chromatography. Similarly, other researchers also conducted residual study of acetamiprid using ethyl acetate as extractant (Ortelli, Edder, & Corvi,

2004). Watts & Storherr (1965) tested collaboratively ethyl acetate, acetonitrile, dichloromethane-water and acetone as extraction solvents for spiked samples by blending, filtration and GC (without clean up). Mukerjee et al. (2007) tested acetone, acetonitrile, and ethyl acetate-hexane in extraction process for mango fortified samples for different pesticides. Mukerjee et al. (2007) used alumina neutral, alumina + florisil and florisil to test recovery percentage for different pesticides in mango for clean up purpose. Various multiresidue methods on acetamiprid determination and extraction have been published for different matrices in the world but the area of pesticide residue study in agricultural commodities is still lacking in Pakistan. Further, already published methods are multi residue in nature and non-specific to single pesticide in fruits and vegetables. So, keeping in view all these aspects, the current study was conducted to monitor the acetamiprid residues in different fruits and vegetables.

2. Materials and methods

A local market basket survey was made at districts Sheikupura and Gujranwala and thirty samples of fruits (apple, and mango) and vegetables (tomato, green chillies, and cucumber) were procured. One kg of each commodity was purchased in accordance with standard procedure (FAO/WHO, 1982) and stored at -4ºC.

The samples were sliced and homogenized. All the samples were spiked with known quantity (0.5 mg/kg) of acetamiprid reference standard (Ehrenstorfer GmbH, acetamiprid 99.0%). Five sub samples of 50 g were subjected to extraction, (using ethyl acetate, alone and dichloromethane+acetone 8:2) and clean up (using different adsorbents i.e. activated charcoal and florisil).

After going through the procedures mentioned below, the samples along with control samples were analyzed with HPLC-PDA comprising of; Light source: deuterium lamp: wavelength 254 nm, pressure 2000-2400 psi, column: C_{18} (ODS)-15cm*6.0mm i.d., stainless steel, injection loop: 10uL, and data acquisition was taken with 3D data workstation. The flow rate of mobile phase (methanol: water/ 60:40) was 1mL/min in isocratic mode. (Parveen et al., 2005).

All the samples were prepared by using ethyl acetate (HPLC grade), acetone (HPLC grade), dichloromethane (HPLC grade), sodium sulphate anhydrous (technical grade), activated graphitized charcoal,(activated the charcoal by heating it at 650ºC for 4 hours in a muffle furnace and then transferred to oven at 130ºC and allowed

Table 1. Testing efficiency of solvents in extraction process for spiked fruits and vegetables samples (Recovery % ± SD)

Solvents	Active ingredient	*Mango	Apple	Chillies	Tomato	Cauliflower	Cucumber
Ethyl acetate	acetamiprid	90±4.0	95±2.0	93±3.0	96±2.0	96±1.0	90±3.0
Dichloromethane + acetone8:2	acetamiprid	85±2.1	89±1.1	86±2.0	88±1.2	90±2.1	84±1.0

Table 2. Efficiency of recovery using different adsorbents (Recovery % ± SD)

Adsorbents	Active ingredient	Mango	Apple	Chillies	Tomato	Cauliflower	Cucumber
Activated charcoal(alone)	acetamiprid	73±2.1	73±4.0	71±1.1	73±2.0	72±3.1	72±3.0
Activated florisil(alone)	acetamiprid	78±3.0	73±3.2	73±2.0	74±2.1	75±1.2	70±4.0
Activated charcoal &florisil	acetamiprid	90±3.6	82±5.6	88±2.1	89±1.2	90±1.0	86±4.1

Table 3. Efficiency of recovery using different eluting solvent (Recovery % ± SD)

Solvent	Active ingreident	Mango	Apple	Chillies	Tomato	Cauliflower	Cucumber
Dichloro methane+acetone(8:2)	acetamiprid	88±2.0	87±4.1	90±1.0	92±1.0	95±1.2	95±1.0
Ethyl acetate	acetamiprid	81±3.2	84±3.0	81±4.0	82±3.0	83±3.0	84±2.1

*n=5 each vegetable and fruit.

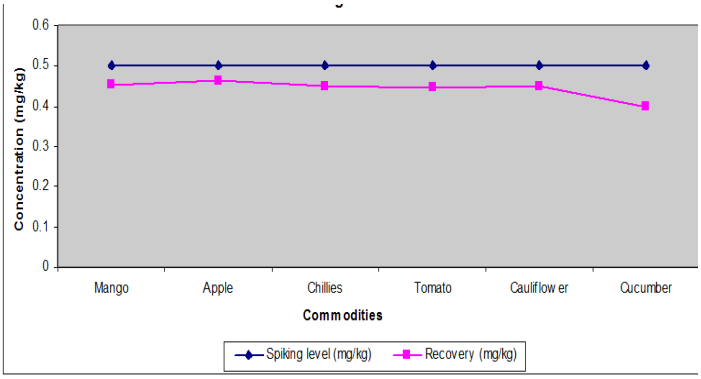

Figure 1. Recovery of acetamiprid in different fruits and Vegetables

to stand for 5 hours and stored in air tight desscicator), activated florisil (60-80 mesh),(activated the florisil by heating it at 650ºC for 4 hours in a muffle furnace and then transferred to oven at 130ºC and allowed to stand for 5 hours and stored in air tight desscicator), sodium chloride (extra pure grade), ultra-pure water was prepared by passing distilled water through Labconco water pro purification system. The non edible portion of the samples were removed and washed with tap water and chopped without peeling. A 500 g of the chopped samples were blended /homogenized at high speed using waring commercial laboratory blender, (Torrington, Connectlut 06790 Assembled in USA). Fifty gram of homogenized sample of each commodity was taken in 250mL conical flask and spiked at 0.5 mg/kg by 100µL of reference standard of acetamiprid.

The spiked samples were allowed to stand for three hours before extraction to test percent recovery of extracting solvents, adsorbents and the eluting solvents (Zahida & Masud, 2002). The samples were extracted by adding 75 mL of ethyl acetate along with 20 g anhydrous sodium sulphate and 25 g of extra pure sodium chloride and blended at high speed using waring blender for three minutes. It was allowed to settle and filtered the supernatant solution through 0.45µm filter paper through vacuum filtration assembly. The solid residue was again blended by adding 25mL of ethyl acetate twice for three minutes and collected the three filtrates into the same flask (Kadenczki, Arpad, & Gardi, 1992; Atif et al., 2007 and Gambacorta et al., 2005). The filtrates from the above were subjected to clean up over activated charcoal and florisil separately and activated charcoal and florisil in tandem. Glass column (30cm length, 1.5 cm i.d.) was packed with 10 g activated florisil and 5 g anhydrous sodium sulphate on the top by plugging glass wool at the bottom of the column (Mukerjee et al., 2007 and Philip et al., 2003). The residue analysis was done using *Alliance* HPLC system of Waters Company.

3. Results and discussion

Primary evaluation of different solvents was made for the extraction of acetamiprid residue from spiked fruits and vegetables samples. The ethyl-acetate was observed an effective extracting solvent alone which produced 90-96% (± 3.0) recovery for acetamiprid. However, another extractant, i.e. dichloromethane + acetone (8:2) gave recovery 84-90 % (± 1.5) (Table 1). Ortelli, Edder, & Corvi (2004) and Kadenczki, Arpad, & Gardi (1992) also showed similar properties of ethyl acetate in term of recoveries and cleanliness and selected it due to its lower toxicity as compared to dichloromethane. Similarly, Ferrer et al. (2005) recovered acetamiprid residues in fruits and vegetables using ethyl acetate alone as extractant.

The extract was imperiled to clean up column through activated charcoal and florisil separately and activated charcoal and florisil in tandem. The results revealed that activated florisil (10g) and activated charcoal (10g) separately was not sufficient for the removal of inter-fearing compounds as a cleanup reagent (Table 2). The maximum percent recovery (82-90% ± 4.6) was noted with the use of activated charcoal (10g) and activated florisil (10g) in tandem (first from charcoal and then through florisil) for acetamiprid residues. Ripley et. al. (2001), Hirostaka et al. (2001), Mukherjee et al. (2007) and Nakamura et al. (1993) found maximum recovery using similar type of column for clean up purposes for residual analysis of acetamiprid in fruits and vegetables.

When a combination of dichloromethane + acetone (8:2) was compared with ethyl acetate as eluting solvents, it produced high recoveries in the range of 87-95% (± 2.7) where as ethyl acetate alone produced 81-84 % (± 3.1) recoveries (Table 3). Mukherjee et al. (2007) reported similar results that dichloromethane + acetone (8:2) gave higher recoveries in the range of 88.6-96.6% in mango fruit as compared to ethyl acetate. Lower percent recovery with ethyl acetate may be attributed to the fact that complete removal of ethyl acetate before analysis by HPLC-PDA detection proved to be tedious due to the presence of trace amount of acetic acid present in it (Ortelli, Edder, & Corvi, 2004). The matrix effects differ from matrix to matrix and pesticide to pesticide. The results given in Table. 2 indicated that the recovery of

acetamiprid residues was independent of the samples matrix. Similar results were reported by Kadenczki, Arpad, & Gardi (1992). The developed method is unique in nature as it is simple and specific for determination of mentioned pesticide in fruits and vegetables and can be used as a multi residue method.

4. Conclusions

Amongst the established extracting solvents, ethyl-acetate was found an effective single solvent which gave recovery 90-96% (± 3.0) for acetamiprid. While among the eluting solvents, a combination of dichloromethane + acetone with 8:2 ratio proved best in yielding high recoveries, i.e. 87-95% (± 2.7). However, activated charcoal and florisil in tandem yielded higher recoveries (82-90 % ± 4.6) among the adsorbents used for clean up.

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