Method of Analysis Establishment and Determination of Benzimidazole Residues in Fruits and Vegetables

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ABSTRACT

A method was developed and validated for easy and simple determination of carbendazim and thiabendazole residues in fruits and vegetables. Most of the validation items; recovery %, limit of quantification, linearity, repeatability, reproducibility, calibration levels and measurements uncertainty were considered.

A total of two hundred samples of different commodities of fruits and vegetable were analyzed for carbendazim and thiabendazol residues through a national monitoring program. Samples were taken from nine different representative governorates in Egypt through the years 2007-2008. The study revealed that about 60 of the samples were free from Carbendazim and 95 samples have a limit which was less than that of quantification and about 15% of the collected were contaminated with carbenazim with concentration level ranged from 0.1-0.81 mg/kg with 90th percentile 0f 0.2 mg/kg. Only 11 samples were contaminated with thiabenazole out of 200 samples in citrus samples and no thiabendazole violation for Codex and European maximum residue limits.

INTRODUCTION

Benzimidazole compounds fungicides are widely used in agriculture for both field and post-harvest treatments. The main compounds in use are thiabindazole (TBZ), benomyl, carbendazim and thiophanate methyl (TFM). As the last three compounds are inter-correlated, having MBC as common metabolite and major fungitoxic principle, a single maximum residue limit (MRL) is generally set for these compounds of this group. Benzimidazole fungicides are systemic and they widely used for controlling fruit and vegetable pathogens (Papadopoulou, 1991).

Benzimidazole fungicides in food are considered to be of significant health risk (Banks and Soliman,1997; Urani et al., 1995; Attilio, 2004). Therefore validation for method of analysis should be established for fruit and vegetables samples to detect the residues of these fungicides, As well as surveillance of residue limits in fruit and vegetables should be done.

Key words: Pesticides, validation, residues, monitoring and bezimidazol.

MATERIAL AND METHOD

Reagents , glassware and apparatus

The used chemicals were ethyl acetate (pestiscan), 0.1 N HCl , 0.1 N HCL aqueous solution saturated with ethyl acetate, 1 N NaOH aqueous solution (Merck), conc. ammonia solution ca. 25% NH_3 (Riedel- de-Haen), 2 N NaOH aqueous solution (Merck) and saline solution (33 g sodium acetate + 200 g sodium chloride dissolved in 1distilled water).Buchner flasks, funnels, separatory funnels (100ml), pipettes (10,5and 2 ml), blender, graduated cylinders (100ml) and rotary evaporator were used for this study .

UV Spectrophotometer UNICAM-UV/VIS Spectrophotometer software Unicam UV2-100 V3.50 for this residues determination provided with Silica cells 2 cm thickness was used.

EXPERIMENTAL

Fifty grams of homogenized fruit or vegetable sample are blended with 100 ml ethyl acetate + 5 ml ammonia for 2 minutes, the filter cake is extracted twice with 50 ml ethyl acetate in blender for 1 min and filtrated on the same vacuum flask. Tne blender jar was washed with additional 50 ml with ethyl acetate. The problems associated with the analysis of this class of compounds have been dealt with Sherma (1975) and Gorbach (1980). Benzimidazole is the parent substance of a family of a systemic fungicides, including benomyl and thiabendazole. If one of the hydrogen atoms of the amino group of carbamic acid is replaced by the benzimidazole redical benzimidazolyl carbamate is formed, the methyl ester of which is called MBC or carbandazim.

In almost all methods, benomyl is readily converted during the analytical procedure into MBC (carbendazim) by diluted acid treatment, either in the extraction stage (Bicchi et al., 1989) when hydrophilic extraction solvent is used, or later in the procedure by shaking the solvent containing the analyte with a diluted acid solution in a separating funnel.

An accurate transfer was done into a 250 ml flask, and the vacuum flask was washed three times with 12 ml ethyl acetate, the combined filtrates were washed and concentrated to 50 ml.

The 50 ml of extract was transferred into a separating funnel and washed with 20 ml 1N NaOH followed by 5 ml de-ionized water. The organic layer and extract were taken with 0.1 N HCL three times successfully (10 ,10,5ml), the three layers were combined together and re-extracted again with 10 ml with a mixture of NaOH, ethyl acetate, saline solution (5,12,5ml). The organic and washes layers were collected again with 10 ml de-ionized water and then extracted with 10 ml 0.1 N HCL saturated with ethyl acetate. The

HCL layer(10ml) was taken for measuring thiabendazole. The aquous layer was re-extracted with a mixture of 5 ml (2 N NaOH)+12 ml (Ethyl acetate), and then that layer was washed as previously mentioned with 10 ml 0.1 N HCL saturated with ethyl acetate . Both tubes of 1st and 2nd extraction were collected together for carbendazim measurement.

Residues Determination

The samples were determined by scanning the spectrum from 250 to 400 nm. Thiabendazol is measured at 302 nm and carbendazim is measured at 279, 282 and 295 nm using 0.1 HCl as a blank for carbendazim and that HCL saturated with ethyl acetate was used as a blank for thiabendazol. A calibration curve was constructed using five levels 1,3,5,7,and 10 µg and the minimum correlation coefficient was r=0.9999. A dilution may be necessary to reduce the maximum absorbance at 302 nm when the total contents of thiabendazol exceeds 10 µg/ml in the final analyzed solution.

RESULTS AND DISCUSSIONS

Method validation summary

Recovery tests

The recovery percentage and repeatability of the running method tested on fruits and vegetables are show in Table 1.

Table (1) The average recoveries of different spiking levels and there corresponding coefficient of variation.

Fungicides	Commodity	Spiking level (µg/kg)	No. of Replicates	Average recovery%	CV %
Thiabendazole	Tomato	0.1	7	93	8
	Apple	0.2	18	88	16
	Orange	0.2	9	92	4.9
	Green beans	0.2	11	87	4.9
	Tomato	1	6	90	6
	Tomato	4	5	80	1.4
Carbendazim	Apple	8.0	6	84	4.23
	Tomato	5.0	5	82	3.45
	Orange	0.2	9	79	7.46
	Green beans	0.2	11	89	7.46

Limit of quantification(LOQ):

The limit of quantification was estimated by measuring the recovery and relative standard deviation at 0.1 mg/kg thiabendazole level in tomato, orange and green beans shown in Table 2.

Table (2) Recoveries percentages and there corresponding CV %.

Compound	Commodity	LOQ(mg/kg)	No.of Replicates	Recovery%	CV%
Thiabendazol	Tomato	0.1	7	93	7.68
	Orange	0.1	6	107	4.04
	G.beans	0.1	6	96	9
Carbendazim	Apple	0.2	12	82	6.66
	Cucumber	0.2	12	84	5.69
	Tomato	0.2	12	84	4.43
	Orange	0.2	6	78	3.6
	G. beans	0.2	6	72	4

Repeatability:

The closeness of agreement between successive measurements of the same measured amples was carried out in the same conditions of measurements were stud ed , and done by spiking a blank sample with 6 times with LOQ levels and the relative standard deviations for thiabendazole and carbendazim were 4 04 and 3.6% respectively.

Reproducibility:

The closeness under conditions, where the results are obtained with the same method on identical test items with different operators at different times are presented in Table 3.

Table (3) Repeatability, reproducibility and uncertainty measurements.

Compound	Repeatability			Reproducibility			Combined Uncertainty (Uc)	Expanded Uncertainty 2 x Uc
	No.of samples	Mean (mg/kg	Relative Standard Deviation CV% (Ur%)	No.of samples	Mean (mg/kg)	Relative Standard Deviation CV% (UR%)		
Thiabendazole	6	0.11	4.04	20	0.18	4.9	6.9	14
Carbendazim	6	0.14	3.6	20	0.16	7.46	8.3	17

Uncertainty Measurement:

Each of the separate contributions to uncertainty is referred to as uncertainty component, when expressed as relative standard deviation. An uncertainty component is known as relative standard uncertainty. The total combined standard uncertainty, equal to the positive square root of the sum of the squares of the individual uncertainty components, and the expanded uncertainty is calculated by multiplying the combined uncertainty by a coverage factor (K) for confidence level of 95% as k=2. The studied uncertainty components are summarized in Table 4.

Table (4) Summary of uncertainty components and relative standard uncertainty

Uncertainty component	Relative standard uncertainty %	Source
1-Precision	11.5	From duplicate analysis of real contaminated samples
2- Bias	1.9	Spiked samples
3- Other sample processing	10	Default value from codex guidelines
4-Other standard prep.	0.5	Reference standard preparation
Combined uncertainty	15.4	-
Expanded uncertainty	31	-

Fruits and vegetables:

Sampling

Two hundred samples were collected from nine governorates from January 2007 to December 2008. Samples were taken to represent vegetables and fruits (green beans, green peas, pepper, tomato, potatoes, cucumber, Apple, grapes, oranges, peach, strawberry and many others).

A) Validation of the method of analysis

A validation was done to be sure that the running method is suitable for the analysis of carbendazim and thiabendazol. The limit of quantification for carbendazim and thiabendazol were 0.1 and 0.2 mg/kg which are relatively suitable for comparing with the maximum residue limits (MRLs). The repeatability and reproducibility were less than 20% and the recovery tests were between 70-120% which were accepted according to Eurachem (IUPAC). The linearity was satisfactory up to 10mg/kg. The expanded uncertainty at 95% confidence levels was found to be less than 31% which means that the method is valid for the required analysis.

b) Monitoring of carbendazim and thiabendazol in vegetables and fruits

A study of the possible contamination of the collected representative samples of vegetables and fruits during two years revealed that 64 samples were not contaminated with carbendazim and 95 samples with less than 0.1 mg/kg quantification level. The contamination levels were ranged from 0.1-0.81mg/kg with an

average 0.09 mg/kg and with a 90^{th} percentile (0.2 mg/kg). Only 4 samples were more than maximum residue limits established by Codex Alimentarius Commision (CAC) out of 200 samples, whereas 25 samples were exceeded the maximum residue limits by European countries which were less than 15% of the total studied samples. The contamination in fruits and vegetables may be attributed to the use of benomyl and thiophenate methyl that probably was converted to carbendazim which consequently increase the level of contamination due to the conversion of the mentioned fungicides and carbendazim as well. Only 11 samples were contaminated with thiabendazole ranged from 0.23-3.75 mg/kg which is lower than the maximum residue limit proposed by CAC and European countries and all the contaminated samples were found in citrus samples because thiabendazole is being used as a post harvest application. Also, the study revealed that the most contaminated and violated samples were in the Egyptian leek. Detailed residues determination and comparison between different MRLs established by both CAC and European countries are illustrated in table 5..

Table (5) Comparison between the detected pesticide residues and the maximum residue limits of CAC and EU Limits

Commodity	No. of tested samples	No. of free samples	No. of contaminating samples		Range of residues Found in mg/kg	No.of violating Samples	
			<LOQ	> or = LOQ		CAC Limits	EU Limits
Apple	5	3	2	-	-	-	-
Cantaloupe	3	2	1	-	-	-	-
Cucumber	5	3	2	-	-	-	-
Green peas	6	5	1	-	-	-	-
Green beans	19	8	11	-	-	-	-
Egyptian leek	11	2	3	6	0.14-1.53	3	6
Grape	51	1	35	15	0.11-0.74	-	5
Orange	24	22	2	-	-	-	-
Peach	13	-	9	4	0.14-0.62	-	3
Pepper	14	5	5	4	0.10-0.75	-	2
Potatoes	2	2	-	-	-	-	-
Strawberries	35	2	26	7	0.12-0.55	-	7
Tomatoes	4	2	2	-	-	-	-
Lettuce	4	2	2	-	-	-	-
Guava	4	1	2	1	0.22	-	1
Total numbei	200	60	103	37		3	24

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الملخص العربي

تطوير طريقة تحليل وتقصي مستوي متبقيات البنزيميدازول فى الخضروات و الفاكهة

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المعمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيلة في الاغذية

أجريت الدراسة لتطوير واختبار كفاءة طريقة تسمح بتقدير متبقيات مركبات الكاربيندازيم والثيابندازول المستخدمة كمبيدات فطرية مرورا بكافة المتطلبات من كفاءة الاسترجاع وأقل حدود كمية للقياس والخطية وكفاءة الطريقة لكافة خطوات الطريقة ومستويات المعايرة وحساب اللايقين وغيره . تم تجميع ٢٠٠ عية من تسعة محافظات فى مصر من الخضروات والفاكهة لتقدير مستويات التلوث بمبيدي الكاربندازيم والثيابندازول لعامى ٢٠٠٨و٢٠٠٧ . أظهرت النتائج بالنسبة لمركب الكاربيندازيم خلو ٦٠ عينة من تواجد متبقيات المبيد فيها مع تواجد المتبقيات فى ٩٥ عينة بمسوى يقل عن الحد الكمى (٠,١ مجم/كجم) . كما وجد أن حوالى ١٥% من العينات التى تم جمعها تحتوى على متبقيات الكاربيندازيم بتركيز يتراوح بين (٠,١ – ٠,٨١ مجم/كجم) وبهذا تزيد عن الحدود الأوروبية المسموح بها. وقد كانت المتبقيات فى ٤ عينات فقط أعلى من الحد الأقصى المسموح به للمتبقيات والذى تقره لجنة دستور الأغذية. أما بالنسبة لتقدير متبقيات مركب الثيابندازول فقد لوحظ إحتواء ١١ عينة فقط من عينات الموالح التى تم جمعها تحتوى على متبقيات هذا المركب (٠,٢٣ – ٣،٧٥ مجم/كجم) وهذه المتبقيات تقل عن تلك الحدود المسموح بها من قبل لجنة دستور الأغذية أو تلك المسموح بها أوروبيا.