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Spectrophotometric Method for the Determination of Carbendazim in Orange Juice Samples Marketed in Senegal

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Authors' contributions

This work was carried out in collaboration between all authors. Author EHTB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DDT and AC managed the analyses of the study. Authors IS, SS, AM, CG, CD, ID, PAD and MDGS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A spectrophotometric method for the quantitative analysis of carbendazim in orange juice is described here. The analytical method was developed for the determination of fungicide in acetonitrile and 10^{-2} M NaOH aqueous solution, with large linear dynamic range (LDR), low limit of detection (LOD) and limit of quantification (LOQ) values of 0.07-0.7 ng/mL and 0.2-2.3 ng/mL,

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respectively, and small relative standard deviation (RSD) values less than 2%, according to the medium. This spectrophotometric method was applied to the evaluation of carbendazime residues in spiked orange juice, with satisfactory mean recovery values within (80-120%).

Keywords: Spectrophotometric; uv-vis absorption; carbendazim; fungicide; solvent effects; orange juice.

1. INTRODUCTION

The current use of pesticides has greatly improved agricultural yields. These compounds comprise a large number of substances with different levels of persistence which are divided into different groups according to the field of use (insecticides, fungicides, herbicides). However, repeated and sometimes exaggerated application of pesticides may result in extensive contamination of water, air, soil and food of vegetable or animal origin [1-7]. Therefore, the development of simple, precise and selective analysis methods for the detection of pesticide residues is necessary to ensure food safety. Currently, several methods of analysis are used for the detection of pesticide residues in food matrices [4-6]. To this end, we have undertaken a study of carbendazim fungicide by spectrophotometry in orange juice samples marketed in Senegal. Carbendazim is a fungicide of the benzimidazole carbamate, was introduced to control various vegetable and orchard diseases (Fig. 1). Carbendazim has been shown to be the solvolysis product of benomyl [8,9]. The fungicide is very persistent in water, and soils. The studies of carbendazim residues in the environment have shown a variation of half-life time ($t_{1/2}$) ranging from a 1 to 6 months [10,11] where photodegradation could be a potential process of the fungicide destruction. Carbendazim is the most widely used active ingredient as systemic fungicide with both protective and curative activities against a wide range of fungal diseases [12].

Carbendazim is reported as one of the most commonly detected pesticides in fruits. Intense use of the fungicide through crops, against a broad spectrum of pathogens is one of the main causes of its presence in foods at trace level. It is often effected by depositing its residues on the surface of the plants, and which are consequently entrained by absorption within some parts of the plant. In China, for example, residues of carbendazim are found up to the level 1 mg/L in fruits [6]. It is one of the twelve most frequently detected pesticides in EU monitoring programs. In addition, between 2002 and 2003 residues of carbendazim and its metabolites were found in 50 samples of bananas imported into Italy from the Ecuador, Costa Rica and Panama with concentrations ranging from 0.05 mg/kg to 1.1 mg/kg [13]. Banned in the United States, the carbendazim trace was recently discovered in orange juice imported from Brazil by American production brands like Minute Maid and Tropicana [14]. The fungicide is toxic for humans, animals and has adverse effects on the reproductive system [15-18].

Due to the high consumption of fruit juices and the resulting environmental impact, control of residues of carbendazim is of great importance. For the first time, the solvent effect of the carbendazim absorption spectra were investigated, and compared in several media, including acetonitrile, NaOH (10^{-2} mol/L), HCl (10^{-2} mol/L) and distilled water. The spectrophotometric characteristics and the analytical performance were determined and discussed. Afterwards, we applied this analytical method for the evaluation of carbendazim residues in spiked orange juice. The chemical structure of carbendazim is presented in Fig. 1.

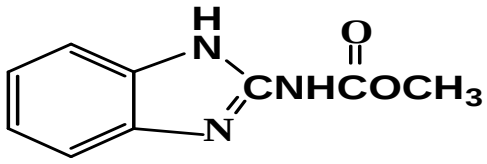

Fig. 1. Chemical structure of carbendazim

2. MATERIALS AND METHODS

2.1 Reagents

Analytical-reagent grade carbendazim (98% m/m) were purchased from Cluzeau Info Labo (Sainte-Foy-la-Grande, France). Sodium hydroxide pellets (97% m/m), hydrochloric acid (37% m/m) and spectroscopic-grade solvent, acetonitrile, were obtained from Sigma Aldrich (Taufkirchen, Germany). Distilled water was used for preparing aqueous solutions of the solute.

2.2 Chemical Composition of Orange Juice Samples

Orange juice samples (Orange MM, Orange TP, Orange RN and Orange PR) were purchased from local markets. The chemical composition of each sample of orange juice as indicated by formulation company (SOBOA Senegal) was presented as follow.

- **Orange MM :** Sugar, citric acid, aroma, ascorbic acid, beta-carotene, arabic gum, glycerol ester of wood resin, maltodextrin, sodium metabisulfite, potassium sorbate, sodium ascorbate.
- **Orange TP :** Sugar, citric acid, aroma, ascorbic acid, beta-carotene
- **OrangeRN :** Sugar, citric acid, aroma,
- **Orange PR :** Sugar, citric acid, aroma.

All orange juice samples studied contain sugar, citric acid and aroma. Only Orange MM and orange TP samples contain ascorbic acid and beta-carotene. Additional compounds such as arabic gum, glycerol ester of wood resin, maltodextrin, sodium metabisulfite, potassium sorbate and sodium ascorbate are also found in the chemical composition of the Orange MM sample. These chemicals compositions of the orange juice samples will be taken into account to explain the difference in the recovery rate obtained after extraction.

2.3 Apparatus

All spectral measurements were performed at room temperature with a UV-vis spectrophotometer, Model Cary 60 UV-Vis. Standard quartz absorbance cuvette with a 1-cm path length (Labo Moderne, France) were used for absorption spectral measurements, and a 20-1000 µL Pipetman micropipette (Gilson, France) was used for dilutions.

The pH and the conductivity measurements were made with a pH-meter Consort C6010 and a conductimeter Hana H198129.

The concentration of the ions and ionic groups were detected using a photometer PF11.

2.4 Solutions Preparation

Stock solutions of HCl (1.0 mol/L) and NaOH (1.0 mol/L) were prepared in 50-mL volumetric flasks with distilled water, and used for serial dilutions. 10^{-3} mol/L stock standard solutions of carbendazim were freshly prepared by exactly weighing and dissolving the fungicide in acetonitrile. All solutions were protected against light with aluminum foil to avoid any decomposition and stored in a refrigerator.

2.5 Preparation of Spiked Orange Juices Samples and Analytical Measure-ments

Samples of orange juice were filtered through a syringe filter PTFE membrane, diameter 25 mm pore size 0.2 µm (Sigma-Aldrich) in order to remove suspended solid matter. Volumes of 10 mL of the orange juice were spiked with a known amount of carbendazim in order to obtain a final concentration C_0 belonging to the linear dynamic range of the calibration graph. An aliquot of carbendazim extracted from juice samples was placed in the quartz absorption cuvette, then the absorption spectra were recorded and the spectra height signal was compare to the standard solution. All absorbance measurements were corrected for the solvent (background) signal with the appropriate blank.

2.6 Solid Phase Extraction (SPE) Procedure

The SPE procedure was used for the determination of carbendazim in spiked orange juice samples. The solid-phase extraction procedure was carried out using a reverse-phase C_{18} cartridge. Firstly, we conditioned the cartridge with 4 mL of acetonitrile, which allowed maintaining it wet. Then, the cartridge was loaded with 5 mL of the spiked orange juices sample, containing 80 or 250 ng/mL of carbendazim. Secondly, the retained pesticide was eluted by 4 mL of acetonitrile. The absorbance was measured and compared to the standard calibration curve in order to determine the carbendazim concentration.

3. RESULTS AND DISCUSSION

3.1 Solvents Effect

The absorption spectra of carbendazim in acetonitrile, distilled water, NaOH (10^{-2} mol/L) and HCl (10^{-2}mol/L) were shown in Fig. 2. Three absorption bands were obtained in acetonitrile at the respective wavelengths of 207, 243 and 287 nm. The absorption spectrum of carbendazim in NaOH (10^{-2} mol/L) show four characteristic bands at 221, 249, 289 and 298

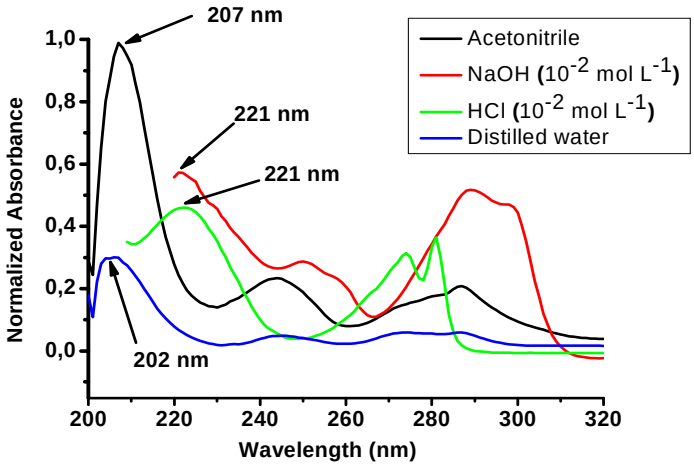

Fig. 2. Normalized absorption spectra of carbendazim ($1.3x10^{-6}$ mol/L) at room temperature in different media

nm with a shoulder which appears at 258 nm. In HCl (10^{-2} mol/L), three absorption bands appear at the respective wavelengths of 221, 274 and 280 nm. For distilled water, carbendazim has three characteristic bands which appear at 202, 280 and 293 nm and a shoulder at 242 nm. The examination of the intensity of these bands reveals that the most intense in acetonitrile is located at 207 nm and for NaOH, HCl and distilled water the maxima appear respectively at 221, 221 and 202 nm.

The molar extinction coefficients (ε_{max}) of these maxima intensity of the bands are 46000, 39500, 24600 and 14000 L/mol.cm respectively for acetonitrile, NaOH, HCl and distilled water. These bands characterize the delocalization of the π electrons of the aromatic nucleus allowing $\pi \rightarrow \pi^*$ transition [19]. On the other hand, the other bands reveal the characteristics of mesomerism noted at the level of the heteroatoms whose delocalization of the π electrons suggests $n \rightarrow \pi^*$ transitions [19]. From an analytical standpoint, in order to gain in rapidity and sensitivity in the spectrophotometric method for the determination of carbendazim, we decided to select acetonitrile and NaOH (10^{-2} mol/L) giving the highest values. In these conditions, the optimal media were acetonitrile and NaOH (10^{-2} mol/L).

3.2 Analytical Figures of Merit

In order to select the optimal analytical conditions, we have optimised our spectrophotometric method for carbendazim determination by comparing the molar extinction coefficients values in the various media under study. Linear calibrations curves of absorbance *vs.* carbendazim concentration were obtained with large linear dynamic range (LDR) and correlation coefficient values (r^2) very close to unity, which indicates a good linearity of the spectrophotometric analytical curves. The limits of detection (LOD) values were calculated on the basis of carbendazim concentration giving a signal-to-noise ratio (S/N) of 3 (IUPAC criterion). The LODs significantly varied with the solvent, ranging from values of 0.07 and 0.7 ng/mL in acetonitrile and NaOH (10^{-2} mol/L), respectively. The limits of quantification (LOQ) values were calculated for S/N ratio of 10, and were comprised between 0.2 and 2.3 ng/mL in acetonitrile and NaOH (10^{-2} mol/L), respectively. The relative standard deviation (RSD) values < 2% indicates the reproducibility of measurements. Table 1 summaries all results.

The difference between the values of LOD in acetonitrile and alkaline NaOH (10^{-2} mol/L) aqueous solution, demonstrated the great analytical interest of utilizing acetonitrile. The LOD value, of 0.07 ng/mL, obtained for acetonitrile by our spectrophotometric method was very low. Therefore, this value suggested that spectrophotometric can be considered as a convenient and sensitive method for determining carbendazim residues in orange juice.

Table 1. Analytical figures of merit for the spectrophotometric determination of carbendazim in orange juice

Solvent	LDR (ng/mL) [a]	LOD (ng mL) [b]	LOQ(ng/mL) [c]	RSD (%) [d]	r^{2} [e]
Acetonitrile	30-2000	0.07	0.2	0.2	0.996
NaOH (10^{-2} mol/L),	46-2200	0.7	2.3	1.8	0.995

[a] Linear dynamic range of the calibration curve; [b] Limit of detection, was defined as the amount of analyte giving a signal-to-noise ratio of 3; [c] Limit of quantification, defined as the amount of analyte giving a signal-to-noise ratio of 10; [d] Relative standard deviation (n = 6); [e] Correlation coefficient

3.3 Analytical Applications to Spiked Orange Juice Samples

To verify the applicability of the proposed spectrophotometric method, carbendazim was determined in spiked oranges juice, after the above-described solid phase extraction procedure. Blank samples were analysed but traces of carbendazim were not found. As shown in Table 2, we found satisfactory recovery values in the case of the various types of oranges juice samples under study, ranging from 80.4% to 119.2%, at different added carbendazim concentrations. These values belong to the validation standards of analytical methods and confirm the effectiveness of the extraction procedure. The standard deviation values were rather small, which indicates a good reproducibility of the spectrophotometric method for analytical applications. The mean recovery values was also dependent on the type of oranges juice sample.

3.3.1 The SPE recovery rate efficiency

The physicochemical parameter associated with these juices samples have an important effect on the carbendazim recovery. At the first time, we remark that the conductivity σ of the juice samples increase with increasing the pH, from orange TP to orange PR samples. At the same time, the recovery rate increase in function of both parameters indicating an optimal pH and/or conductivity values suitable for recovery determination: these optimal values are 3.17 for the pH and 1085 µS/cm for the conductivity. On the other band, the organic chemical compound added by the different manufacturers in these juices have no significant effect in the recovery rate as shown by orange RN and orange PR which have similar composition but different recoveries. The same remark can be made by comparing the samples of orange MM and orange TP which have four components in common, but orange MM with more additives, has a better recovery than orange TP. We can conclude that the only parameters that have an effect difficult to eliminate by SPE extraction and that act on the recoveries are the acidity and/or the conductivity of the samples.

3.3.2 Effect of ions on the recovery rate

Since the orange juice samples show some ions, we judged it necessary to evaluate the role of these ions in the recovery rates obtained. The effect of several inorganic ions including $PO4^{3-}$, NO^{3-}, $SO4^{2-}$, Cl^{-} and Zn^{2+} on the absorbance was investigated for possible interference on the determination of carbendazim. For this end the concentration of the ions and ionic groups were measured using photometer PF11 which gives directly the concentration of the ion (Table 3). We found that orange juice samples with a high chloride ions concentration such as Orange PR and Orange RN have relatively high recovery

Table 2. Recovery rates of carbendazim determination in orange juice after SPE extraction

Orange juices samples	Added (ng/mL)	Founded (ng/mL)	Recovery (%)	Mean recovery (%)	*σ (µS/cm)	pH
Orange PR	80	100	125.0			
	250	283.5	113.4	119.2 ± 8.2	2561	3.56
Orange RN	80	73.2	91.7			
	250	258.5	103.4	97.5 ± 8.3	1085	3.17
Orange MM	80	72.0	90			
	250	232.5	93.0	91.5± 2.1	1052	2.94
Orange TP	80	59.5	74.4	80.4 ± 8.5		
	250	216.2	86.5		981	2.73

**σ (µS/cm) = conductivity of the juice samples*

Table 3. Measured concentration of some ions or ionic groups

	Orange MM	Orange PR	Orange RN	Orange TP
$PO4^{3-}$ (mg/L)	1.6	< 0.2	< 0.2	< 0.2
NO^{3-}(mg/L)	4	< 4	< 4	< 4
$SO4^{2-}$ (mg/L)	67	-----	-----	-----
Cl^{-} (mg/L)	6	64	55	4
Zn^{2+} (mg/L)	<0.1	< 0.1	0.3	< 0.1

rates when compare to the remaining samples. It can therefore be said that chloride ions probably increase the solubility of the fungicide carbendazim in aqueous medium. The sample (Orange TP) having the lowest value of the recovery rate contain the lowest concentration of chloride ion. The other measured ions do not have a remarkable effect on the recovery rate. Since their concentrations are relatively low.

3.4 Analytical Interest of the Method

The Spectrophotometric method developed for carbendazim determination present several analytical advantages over other methods described in the literature [4,6,21-23]. The method has many advantages, in particular the low costs of equipment, the treatment of the samples, the speed, the sensitivity and the performance. On the other hand, it has certain disadvantages, such as the presence of some organic compounds which absorb [20]. The obtained LOD in acetonitrile is lower than those reported previously for several analytical method of carbendazim determination [22,23]. The main interest of our method is that it is appropriate for the analysis of fungicide residues in orange juice. We have contributed to well define the conditions and to acquire the know-how requested for realizing the carbendazim effective management in orange juice.

4. CONCLUSION

The comparative study of the carbendazim in various media as led us to develop and optimize a reliable, simple and sensitive spectrophotometric analytical method for the determination of carbendazim in acetonitrile and NaOH (10^{-2}mol/L). The main point of interest of this method is that it can be easily applied to carbendazim-treated fields, allowing the quantitative analysis of fungicide residues in an aqueous environment. Excellent analytical results have been obtained confirming the lowest concentrations of carbendazim detected, 0.07 and 0.7 ng/mL in acetonitrile and NaOH (10^{-2} mol/L) respectively. The analytical applications to the determination of carbendazim residues in spiked orange juice have also led to satisfactory recovery values.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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