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# DETERMINATION OF FUNGICIDE RESIDUES IN GRAPE BY GC/MS

LAZIĆ SANJA[1], KOMLEN VEDRANA[2], ŠUNJKA DRAGANA*[1], GRAHOVAC NADA[3], PEJČIĆ JADRANKA[2], RAHIMIĆ ALMA[2], BLESIĆ MILENKO[4]

[1] University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, Novi Sad, SERBIA
[2] University Džemal Bijedić, Agromediterannian faculty, Sjeverni logor bb, Mostar, BiH
[3] Institute for Field and Vegetables Crops, Maksima Gorkog 30, Novi Sad, SERBIA
[4] University of Sarajevo, Faculty of Agriculture and Food Science, Zmaja od Bosne 8, Sarajevo, BiH
*Corresponding author
e-mail address: draganas@polj.uns.ac.rs

The extensive use of pesticides in modern farming on fruit and vegetables has posed risks to public health and environment. Pesticide residues can be found even when they are applied in accordance with good agricultural practices. This paper deals with the analysis residues of three fungicides (benalaxyl, dimethomorph, tetraconazole) by chromatographic method in grape samples. The applicability of the proposed method to detect and quantify pesticide residues has been demonstrated by the analysis sample grapes varieties "Žilavka" and "Blatina" cultivated on a small farm in the vineyards in a broader the region of Mostar. Analytes were extracted using SPE technique and analysis is performed by gas chromatography, employing mass selective detection in the selected ion monitoring mode. The content fungicides (benalaxyl, dimethomorph, tetraconazole) in grape samples is regulated by relevant EU regulation (The Annexes of Commission Regulation (EC), No. 396/2005). In grape samples analyzed in this study residues of benalaxyl and tetraconazole were found. The fungicide concentrations were bellow the MRL value permitted by EU regulations for grape.

*Key words*: grape, fungicides, gas chromatography, residue analysis

## INTRODUCTION

Fungicides represent one of the most relevant groups of pesticides applied to vineyards. These compounds are sprayed directly on fruit and leaves to prevent the attack of fungi, which reduce the yield of fruit (Otero et al., 2003). Residues of these compounds were believed to be one of the most important pollution sources in food production and might pose potential threat to public health. However, if these harmful chemicals are not degraded naturally, they will penetrate plant tissues and appear in the pulp and juice. Once present in the pulp, pesticides are difficult to completely be removed. Moreover, the concentration of pesticide residues may increase during post processing, where in general pesticide concentrations in processed juices are higher than that in the natural fruit (Cabras

and Angioni, 2000). Pesticide residues in fruit wine, like those found in juice, are also introduced from planting and preservation process. Several different fungicides are widely used in the treatment of diseases of grapes (Alawi, 1995; Cabras and Angioni, 2000; Cabras et al., 1997; Cabras et al., 1998). Because of the health risk of pesticide residues in fruit and wine, it is of particular importance to provide precise, accurate and reliable test result of residues as the scientific basis for ensuring food safety and fair practice in international trade. Pesticide residue analysis is becoming one of the most active directions in the field of analytical chemistry. Most of the traditional analysis methods of pesticide residues are applied to detection of a single component or a category of pesticides. On the contrary, multi-residue analysis method can be used to analyze not only different components of same type of pesticides, but also different components of different types of pesticides. The development of multi-residue methods represents a relatively new trend in pesticide residue analysis.

Sample preparation plays an important role in the field of pesticide residue analysis. Solid phase extraction (SPE) is the most common method of extracting fungicides and renders high extraction yields. It has been proven that SPE offered several significant advantages over LLE, such as less consumption of organic solvent, shorter analysis time, no phase emulsion, higher method recovery, and more efficient removal of interfering compounds. SPE can be used to isolate analytes of interest from a wide variety of matrices, including juice and wine. It was often used combined with LLE as means for enrichment and purification. Several multiresidue methods are available for the determination of residues of different triazoles in various food products such as processed fruits, vegetables, grapes, wines and strawberries (Garland et al., 1999; Sannino, 2004; Zambonin et al., 2002) involving intensive sample preparation such as solid phase extraction which is time consuming and labor intensive. The residues of these fungicides are analyzed by gas–liquid chromatography by both nitrogen phosphorous detector (NPD) and electron capture detector (ECD), or by techniques such as gas chromatography-tandem mass spectrometry for confirmation and quantization (Bernal et al., 1997; Otero et al., 2003; Schermerhorn and Golden, 2005; Trosken et al., 2005).

Benalaxyl, dimethomorph and tetraconazole is included among the active substances in Annex I to Directive 91/414/EEC (http://ec.europa.eu/sanco_pesticides) and their structures are shown in Figure 1.

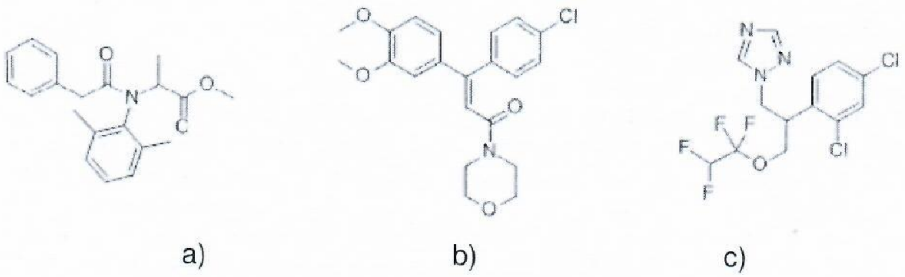


**Figure 1**. Structures of fungicides a) benalaxyl b) dimethomorph c) tetraconazole

The aim of this work was to determine fungicides residues (benalaxyl, dimethomorph, tetraconazole) in grape samples. Residues were determined using a gas chromatograph equipped with a mass selective detector (MSD).

## MATERIAL AND METHODS

The vineyards choice was done to research on four different locations in the vineyards of Mostar. At three locations - Humčine, Mukoša and Žitomislić - it was a plantation vineyards, while the location Hodbina was a small vineyard. The field sampling was conducted during the technological maturity of the grape varieties of "Žilavka" and "Blatina" and concentration of fungicide residues determinated in samples of grape of this varieties. The average sample was taken from five grapevines with different heights and foreign vines. The average weight of each laboratory sample of grapes was approximately 2 kg with at least five clusters. The samples were packed in plastic bags and hand delivered to the laboratory refrigerator (Table 1).

**Table 1.** Place and method of sampling grapes

| Location | Varieties | Number of samples |
|---|---|---|
| Humčine | Žilavka | 4 |
| | Blatina | 4 |
| Mukoša<br>Žitomislić | Žilavka | 4 |
| | Blatina | 4 |
| Hodbina | Žilavka | 4 |
| | Blatina | 4 |

Treatments were carried out air assistance sprayers (atomizer) with 300 l with average interval between treatments was 10 days (the largest gap was 17 days, a minimum distance of applications 3 days) as presented on table 2. During vegetation has been free of disease. The protection program grape in the vineyard plantation Žitomislić was the same as the program was conducted in the vineyard plantation Mukoša (Table 2-4).

**Table 2.** The protection program grape in the vineyard plantation Humčine

| No. | Date treatment | Product | Active substance | Quantity kg or l/ha |
|---|---|---|---|---|
| 1. | April 21st | Folpan | Folpet | 2.05 |
| | | Kossan WG | Sulfur | 4.1 |
| 2. | May 6th | Folpan | Folpet | 2.5 |
| | | Kossan WG | Sulfur | 4.9 |
| 3. | May 14th | Manfil 75 WG | Mancozeb | 3.3 |
| | | Karathane Gold 350 | Meptyldinocap | 0.9 |
| 4. | May 23rd | Manfil 75 WG | Mancozeb | 3.3 |
| | | Karathane Gold 350 | Meptyldinocap | 0.9 |
| 5. | May 27th | Fantic M | Benalaxyl + mancozeb | 4.9 |
| | | Domark 40 ME | Tetraconazole | 1.4 |
| 6. | June 16th | Fantik F | Benalaxyl + folpet | 4.9 |
| | | Crystal | Quinoxyfen | 0.4 |
| 7. | June 29th | Fantikc F | Benalaxyl + folpet | 4.9 |
| | | Crystal | Quinoxyfen | 0.4 |
| 8. | July 15th | Mythos | Pirimetanile | 2.5 |

**Table 3**. The protection program grape in the vineyard plantations Mukoša and Žitomislić

| No. | Date treatment | Product | Active substance | Quantity kg or l/ha |
|---|---|---|---|---|
| 1. | May 5th | Delan | Dithianon | 0.3 |
| | | Sulfur | Sulfur | 1.5 |
| 2. | May 15th | Delan | Dithianon | 0.4 |
| | | Thiovit jet | Sulfur | 2.0 |
| 3. | May 25th | Pergado F | Mandipropamid+ folpet | 2.0 |
| | | Talendo | Proquinazid | 0.25 |
| 4. | June 5th | Fantic F | Benalaxyl + folpet | 2.5 |
| | | Domark, | Tetraconazole | 0.75 |
| 5. | June 18th | Forum star | Dimethomorph + folpet | 2.0 |
| | | Collis | Kresoxim-methyl + boskalid | 0.4 |
| 6. | June 30th | Mikal premium | Fosetyl + folpet+ iprovalicarb | 3.0 |
| | | Postalon | Quinoxyfen + myclobutanil | 0.125 |
| 7. | July 12th | Cabrio top | Pyraclostrobin + methiram | 2.0 |
| 8. | August 9th | Mythos | Pirimethanil | 2.5 |

**Table 4**. The protection program grape in the vineyard plantation Hodbina

| No. | Date treatment | Product | Active substance | Quantity kg or l/ha |
|---|---|---|---|---|
| 1. | April 20th | Cuprablau | Copper | 3.0 |
| | | Chromosul | Sulfur | 2.5 |
| 2. | April 28th | Chromosul | Sulfur | 2.5 |
| 3. | May 7th | Chromosul | Sulfur | 2.5 |
| 4. | May 20th | Ridomil gold | Metalaxyl + mankozeb | 2.5 |
| | | Chromosul | Sulfur | 2.5 |
| | | Topas | Penconazole | 0.25 |
| 5. | June 1st | Ridomil gold | Metalakxyl + mankozeb | 2.5 |
| | | Chromosul | Sulfur | 2.5 |
| 6. | June 11th | Fantic F | Benalaxyl + folpet | 2.5 |
| | | Domark, | Tetraconazole | 0.75 |
| | | Pirus | Pirimetanil | 2.5 |
| 7. | June 24th | Acrobat | Dimethomorph + mankozeb | 2.5 |
| | | Systane 24 E | Myclobutanil | 1.0 |
| 8. | July 4th | Cuprablau | Copper | 3.0 |
| | | Thiovit jet | Sulfur | 4.0 |
| 9. | July 20th | Nordoks 75W | Copper | 1.25 |
| | | Thiovit jet | Sulfur | 4.0 |
| 10. | August 5th | Mythos | Pirimetanil | 2.5 |

In this study we are investigated the presence of benalaxyl, dimethomorph and tetraconazole residues in grape. Fungicides were extracted from homogenized samples of grape by mechanical shaking with acetonitrile (Fillion et al., 2000). Clean-up is necessary in order to reduce the detection limits of the method and/or to avoid interferences from the barley malt matrix. The concentrated sample extracts may contain a high content of co-extractives which can damage the GC column, resulting in a matrix enhancement effect (Hajšlová et al., 1998).

## GC–MS ANALYSIS

Determinations were performed on Thermo type Focus DSQ II gas chromatograph fitted with an mass selective detector. The gas chromatograph was equipped with sampler and split less injector with electronic pressure control. The mass spectrometer was used with electron impact ionization (70 eV) in Scan mode and selected ion monitoring (SIM) mode (Table 5).

**Table 5**. Operational conditions

| | |
|---|---|
| Operating mode | splitless |
| Injection volume | 2 µl |
| Injector temperature | 250 °C |
| Temperature detector | 285 °C |
| Helium flow rate | 0.9 ml/min |
| Initial column temperature | 70 °C |
| Initial time | 2 min |
| Speed of temperature rise | 25 °C/min |
| Final temperature | 280 °C |

Identification of the studied analytes was done by comparing mass spectra and retention times of the samples grape with working standard solutions of fungicides. The identification was confirmed by comparing the relative abundances of ions (quantifier and qualifiers) of the experimental standards against well-known relative abundances of the US National Institute of Standards and Technology (NIST) library reference spectra. The mass spectrometer was calibrated with perfuorotributylamine (PFTBA).

Limit of detection was calculated using Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry (MacDougall and Crummett, 1980).

## RESULTS

Under the selected conditions, the linearity of the calibration curve was evaluated in a concentration range. LOD was 0.001 mg/kg. Average value for the recovery of benalaxyl, dimethomorpf and tetraconazole were > 90%. The final extracts of grape samples made by the proposed SPE method were satisfactorily clean for direct GC-MS analysis (Figure 2). The results of fungicide residues in the grapes are shown in the Table 6.

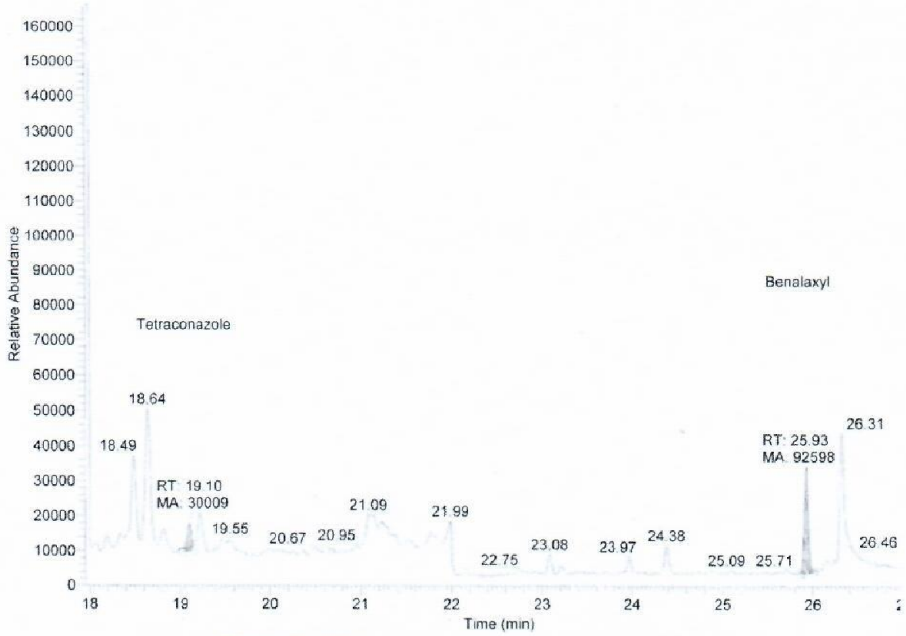


**Figure 2**. GC-MS chromatogram of grape samples

**Table 6**. Determined value of fungicides in grape by GC-MS

| Locality | Variety | Investigated compound | Determinted value (mg/kg) | LOD* (mg/kg) |
|---|---|---|---|---|
| Humčine | Žilavka | Benalaxyl | < LOD | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | 0.003 | 0.001 |
| | Blatina | Benalaxyl | 0.014 | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | 0.017 | 0.001 |
| Mukoša | Žilavka | Benalaxyl | 0.002 | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | 0.003 | 0.001 |
| Žitomislić | Blatina | Benalaxyl | < LOD | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | 0.001 | 0.001 |
| Hodbina | Žilavka | Benalaxyl | < LOD | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | 0.002 | 0.001 |
| | Blatina | Benalaxyl | < LOD | 0.001 |
| | | Dimethomorph | < LOD | 0.001 |
| | | Tetraconazole | < LOD | 0.001 |

*LOD- Limit of detection

In samples of grape, variety Žilavka, residues of tetraconazole were found in samples from all localities (Humčine, Mukoša and Hodbina) in amount of 0.002-0.003 mg/kg, while benalaxyl was found only in samples from locality Mukoša (0.002 mg/kg).

The residues of benalaxyl in grape, variety Blatina were found in samples collected from locality Humčine (0.014 mg/kg). Fungicide tetraconazole in the same samples were found in amount of 0.001-0.017 mg/kg (localities Humčine and Žitomislić).

The residues of tetraconazole found in grape samples from field experiments were clearly below the EU established MRL values (0.5 mg/kg), thus causing no problems in food safety. The residues of benalaxyl were also below EU MRL (0.3 mg/kg). Residues of dimethomorph were not found in analyzed grape samples.

## DISCUSSION

A number of analytical methods designed to determine multiple pesticide residues have been developed since the 1950s and 1960s and have greatly contributed to agricultural productivity (Jeong et al., 2012). Chromatographic methods represent the most suitable approach for determining pesticide residues in food samples (Schenck et al., 2002). In this work fungicide residues from grape samples were isolated by SP extraction with acetonitrile and determination was performed using GC/MS. Considering high value of the recovery (>90 %), applied method was completely suitable for this kind of analysis.

The highest value of the fungicide residues were found in grape variety Blatina from the locality Humcine. It is can be explain with intensive application of fungicide benalaxyl on this locality (Table 2). The content of fungicide residues were under MRL permitted from European Union for those selected pesticides.

The obtained results it can be concluded that the applied fungicide in grape samples naturally degraded and ensure their safety for safe use. The obtained positive results for tetraconazole and benalaxyl could be attributed to the high sensitivity of the developed GC–MS method.

The main reasons that the amounts of the residues were bellow the maximal residue limits are considering of appropriate fungicide concentrations at the application and also considering of safety intervals, which have to run out from the last application and harvest (Čuš et al., 2011).

## CONCLUSIONS

In this research, SPE combined with GC-MS has been applied to the simultaneous determination of selected fungicide residues in grape samples. Performance of the proposed method fits the requirements for the determination of selected fungicides in real grape samples. Using MSD, quantification (through selective ion monitoring) and confirmation are achieved simultaneously. Residues of dimethomorph in analyzed samples of grape, variety Žilavka and Blatina were not detected. The concentrations of benalaxyl and tetraconazole in the analyzed samples of grape were 0.002-0.014 mg/kg and 0.001-0.017 mg/kg, respectively. These results were below the permissible levels (MRL) set by EU regulations (The Annexes of Commission Regulation (EC) No 396/2005) for tetraconazole and benalaxyl in grape.

## REFERENCES

Alawi, M. A. (1995): Development of a New Approach for the Determination of Folpet in Grapes. Analytical Letters, 28(2): 349–356.

Bernal, J.L., Del nozal, M.Z.M.J., Jimenez, J.J., Rivera, M.J. (1997): Matrix effects in the determination of acaricides and fungicides in must by gas chromatography with electron capture and nitrogen phosphorus detection. Journal of Chromatography A, 778(1–2):111–117.

Cabras, P., Angioni, A. (2000): Pesticide residues in grapes, wine, and their processing products. Journal of Agricultural and Food Chemistry, 48(4): 967–973.

Cabras, P., Angioni, A., Garau, V. L., Minelli, E.V. (1997): Gas chromatographic determination of cyprodinil, fludioxonil, pyrimethanil, and tebuconazole in grapes, must, and wine. Journal of AOAC International, 80(4): 867–870.

Cabras, P., Angioni, A., Garau, V.L., Pirisi, F.M., Brandolini, V. (1998): Gas chromatographic determination of azoxystrobin, fluazinam, kresoxim-methyl, mepanipyrim, and tetraconazole in grapes, must, and wine. Journal of AOAC International, 81(6): 1185–1189.

Čuš, F., Čadež, J.N., Raspor, P.I. (2011): Fungicide Residues in Grapes Determined the Dynamics of *Saccharomyces cerevisiae* Strains during Spontaneous Wine Fermentation. Proc. Nat. Sci, Matica Srpska Novi Sad, № 121: 85—101.

EU Document No. 3010, Status of active substances under EU review (http://ec.europa.eu/sanco_pesticides).

Fillion, J., Sauve, F., Selwyn, J. (2000): Multiresidue Method for Determination 251 Pesticides in Fruits and Vegetables by GC/MS and HPLC/Fluorescence. Journal of AOAC International 83: No3, 698-713.

Garland, S., Menary, R., Davies, N. (1999): Dissipation of propiconazole and tebuconazole in peppermint crops (Mentha piperita) (Labiatae) and their residues in distilled oils. Journal of Agricultural and Food Chemistry, 47(1):294–298.

Hajšlová J., Holadová K., Kocourek V., Poustka J., Godula M., Cuhra P., Kempný M. (1998): Matrix-induced effects: a critical point in the gas chromatographic analysis of pesticide residues. Journal of Chromatography A, 827: 283-295.

Jeong, I.S., Kwak, B.M., Ahn, J.H., Jeong, S.H. (2012): Determination of pesticide residues in milk using a QuEChERS-based method developed by response surface methodology. Food Chemistry, 133 :473–481.

MacDougall, D., Crummett, W.B. (1980): Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry. Analytical Chemistry, 52: 2242–2249.

Otero, R.R., Granade, B.C., Gandara, J.S. (2003): Multiresidue method for fourteen fungicides in white grapes by liquid-liquid and solidphase extraction followed by liquid chromatography-diode array detection. Journal of Chromatography A, 992(1–2):121–131.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. (http://ec.europa.eu/sanco_pesticides/public/index.cfm)

Sannino, A. (2004): Evaluation of a method based on liquid chromatography/electrospray tandem mass spectrometry for analyzing eight triazolic and pyrimidine fungicides in extract of processed fruits and vegetables. Journal of AOAC International, 87(4):991–996.

Schenck, F. J., Lehotay, S. J., & Vega, V. (2002). Comparison of solid-phase extraction sorbents for cleanup in pesticide residue analysis of fresh fruits and vegetables. Journal of Separation Science, 25:883–890.

Schermerhorn, P.G., Golden, P.E. (2005): Determination of 22 triazole compounds including parent fungicides and metabolites in apple, peaches, flour and water by liquid chromatography, tandem mass spectreometry. Journal of AOAC International, 88(5):1491–1502.

Trosken, E.R., Bittner, N., Volkel, W. (2005): Quantitation of 13 azole fungicides in wine sample by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 1083(1–2):113–119.

Zambonin, G., Cilenti, A., Palmisano, F. (2002): Solid-phase micro extraction and gas chromatography-mass spectrometry for the rapid screening of triazole residues in wine and strawberries. Journal of Chromatography A, 967(2):255–260.