

## ADVANTAGES AND DISADVANTAGES OF ACTIVE CARBON IN QuEChERS SAMPLE PREPARATION METHOD FOR PESTICIDE RESIDUES

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### Abstract

*QuEChERS is an analytical method which simplifies the the sample preparation for pesticide residues. It is the result of the necessity to save the time for sample preparation, to reduce the amount of toxic organic solvents and thereby to contribute to the preservation of the environment. In order to obtain a higher recovery, thus making the analysis results more precise and representative, it is necessary during the extraction to pay particular attention to matrix components which can significantly affect the investigation results. In order to decrease the effect of the present components it is necessary to use the adequate substances – sorbents and to examine how their presence affects the validation parameter. The sour cherry extract, as an exceptionally pigmented matrix, needs the use of a sorbent with a strong affinity towards planar molecules thus causing its discoloration by removing the pigments from the extract. The most frequently used sorbents in QuEChERS method of extract purification are primary secondary amine (PSA), graphitized black carbon sorbent (GCB), C18 and Z-Sep sorbent (silicon dioxide coated by zirconium). The paper deals with a possibility of using active carbon (AC) as a possible sorbent which presents a form of carbon obtained in controlled oxidation processes having a porous structure with spacious active surface which enables it to adsorb a wide range of compounds and pollutants from the extract. By use of AC and QuChERS methods an exceptionally purified colorless extract is obtained. The obtained validation parameters point at a very low recovery of the studied pesticides which is a serious disadvantage of this sorbent while high correlation coefficients ( $R^2 > 0.99$ ) are obtained with irrelevant matrix effect in the linearity investigation process.*

**Key words:** AC sorbent, QuEChERS, validation, pesticide residues, LC-MS/MS.

### INTRODUCTION

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is an acronym for the analytical method which, to a great extent, simplifies the analysis of pesticide residue determination in the samples of various origin. It is the result of the necessity to save time for sample preparation, decrease the amount of toxic organic solvents and to contribute to the preservation of the environment (Anastasiades et al., 2003). QuEChERS method in its basic form comprises two steps stages: liquid micro extraction by use of acetonitrile as a solvent with the addition of salts aimed at a better phase separation line and the adjustment of pH values; matrix purification using sorbents.

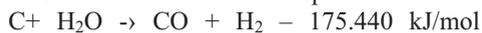
The sour cherry extract, as an exceptionally pigmented matrix, needs the use of a sorbent with a strong affinity towards planar molecules

thus causing its discoloration by removing the pigments from the extract (Bursić et al., 2013).

The extraction of pesticides from plant tissues is a complex task. The process of proving and determining particular pesticides depends on the present compounds in the matrix which can interfere with their separation. Acetonitrile (MeCN) is the most frequently used solvent in the extraction. In the QuEChERS method MeCN is used together with the combination of salts NaCl and MgSO<sub>4</sub> which help with better phase separation and contribute to the recovery without dilution. In the phase of extract purification the most frequently used sorbents are PSA (primary secondary amine), AC (active carbon), GCB (graphitized black carbon sorbent), C<sub>18</sub> sorbent, Z-Sep sorbent (silicon dioxide coated by zirconium) and as the latest EMR-Lipid (Bursić et al., 2016). Their function is to remove fatty acids, sugars and lipids from

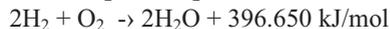
the matrix that makes them quite suitable for the purification of various matrices.

In order to reduce the matrix influence on the detection of pesticide residues at LC-MS/MS sour cherry analysis the substances which have a strong affinity towards planar molecules and efficiently remove the extract pigments are used. The objective of the study is to check the linearity and recovery of pesticides from sour cherries by use of liquid chromatography with tandem mass spectrometry (LC-MS/MS) in case when for the extract purification, within the QuChERS method, AC is used as a sorbent. AC is a form of carbon obtained in carefully controlled oxidation processes resulting in its porous structure. There are three different pore groups: macro-pores (radius > 25 nm); mezzo-pores (radius 1-25 nm) and micro-pores (radius < 1 nm). The most numerous are macro-pores which attract small gas molecules and mezzo-pores which attract larger molecules like color molecules (Momčilović, 2012). The functional groups on the surface of active carbon make it amorphous so that, depending on the pH values of the solution and the adsorbents, the mechanism can be based on the dipole-dipole interactions, hydrogen and covalent binding or ion exchange. The basic units of the active carbon composition are micro crystals consisting of planes of joint hexagonal carbon rings. The functional groups of heteroatoms on the edges of aromatic layers disturb the parallel orientation of planes characteristic of graphite and provide so-called turbostratic structure typical of active carbon. The most frequent heteroatoms in the structure are hydrogen, nitrogen, sulphur and phosphorus and the functional group which they make determine the reactivity in adsorbent, catalytic and electrochemical processes. The given AC structure presents a large active surface that enables it to adsorb a wide range of compounds and pollutants from particular environments. The active carbon can be obtained from various raw materials which contain a high percentage of carbon such as wood, coal, coconut shells. The thermic decomposition of these raw materials is performed at the temperature of 900 °C combined with water vapour:



The reaction is exothermic and the temperature

is sustained by partial burn of the products of carbon-monoxide and hydrogen.



The air is proportionally added to the system in order to burn the gases without burning the carbon.



Figure 1. AC porous structure

The objective of the paper is to study the effect of AC, as a sorbent, in the process of extract purification by QuChERS method to basic validation parameters of multi-residual LC-MS/MS method for the determination of pesticide residues in sour cherries using carbofuran-D3 as an internal standard.

## MATERIALS AND METHODS

**Chemicals:** The analytical standards of all the studied pesticides are the products by Dr. Ehrenstorfer. The stock and the working solution were prepared by dissolving pesticides in methanol (HPLC purity, J.T. Baker).

**Validation parameters:** The recovery was checked for the levels of 0.05 and 0.1 µg/ml in three replications. To 10 g of the control sample 100 µg/ml of internal standard and 100 (50) µl of working solution of mass concentration was added so that final pesticide concentration in the enriched sample was 0.1 (0.05) µg/ml. The linearity of the detector response was performed for the concentration of 0.2; 0.1; 0.05 and 0.02 µg/ml by adding 1 ml of the already prepared calibration standards to the evaporated extract. The matrix effect (ME) was calculated based on the slopes of the calibration curves in matrix and solvent (SANCO/12571/2013).

**Instrument:** An Agilent 1200 (Agilent Technologies, USA) system with a binary pump and autosampler was used. This was equipped with a reversed-phase C18 analytical

column of 50×4.6 mm and 1.8 μm particle size (Agilent Zorbax Eclipse XDBC18). Mobile phases were 0.1% formic acid in methanol (solvent A) and 0.1% formic acid in Milli-Q water (solvent B). The gradient was 0 min (80% B), 10 min (50% B), 20 min (5% B), 24 min (0% B), 25 min (80% B), with the flow rate 0.4 ml/min. For the mass spectrometric analysis, an Agilent 6410B Triple-Quad LC/MS system was applied. Agilent MassHunter software was used for method development, acquisition and data processing.

## RESULTS AND DISCUSSIONS

Before the calibration and quantification of pesticides it is necessary to set the acquisition parameters of mass spectrometer- to determine the reactions for ion monitoring (MRM), to find the energy of collision cell (CE) and the fragmentation energy (Frag) where the response of the studied pesticide will be the highest for the given conditions (Table 1). The

recovery of the studied pesticides was checked for the levels of 0.05 and 0.01 μg/kg. The control of the detector response linearity was carried out for a series of mass concentrations: 0.02; 0.05; 0.1 and 0.2 μg/ml.

The average values of the recovery from sour cherry matrix as well as the correlation coefficients ( $R^2$ ) with the ME are shown in Table 1.

By use of AC in the purification process of sour cherry extracts the exceptionally clear extracts are obtained with the high  $R^2$  values (>0.99) which entails the insignificant ME on all the studied pesticides. Namely, according to SANCO/12571/2013 regulation the assessment of ME on the studied pesticides i.e. their ionization in the ion source can be regarded as being of no influence in case ME is within the interval from 0 to ±15%, if the values range from ±15 to 25% the influence is slight and if the values exceed ±25% ME is significantly prominent.

Table 1. Pesticides analyzed by LC-MS/MS and some of their MS and analytical performance characteristics

Pesticide	MW <sup>1</sup>	MRM transition (m/z)	Frag. <sup>2</sup> (V)	CE <sup>3</sup> (V)	Rt (min) <sup>4</sup>	$R^2$ <sup>5</sup>	ME (%) <sup>6</sup>	Average Rec (%)	RSD <sup>7</sup> (%)
Carbendazim	191.20	192.1→160.1 192.1→132	104 104	18 34	7.869	0.9999	-0.42	16.6	4.27
Pyrimetaniil	199.25	200.1→107.1 200.1→82.1	136 136	26 30	15.050	0.9989	-0.52	32.2	5.31
Azoxystrobin	403.39	404.1→372.1 404.1→344.1	100 100	9 25	15.964	0.9991	-0.13	26.0	7.34
Metoxyfenozide	368.47	369.2→149.1 369.2→313.2	100 90	12 15	16.599	0.9994	0.17	20.0	2.95
Myclobutanil	288.78	289→125.1 289→79.2	150 150	20 15	16.669	0.9999	11.09	32.6	3.58
Cyprodinil	225.29	226.1→93 226.1→108	120 120	30 40	16.678	0.9974	1.49	20.6	7.94
Triadimenol	295.76	296.2→227 296.2→70.2	60 60	5 10	16.887	0.9988	11.86	30.2	6.07
Fenhexamide	302.20	302.1→97.1 302.1→55.1	110 110	25 30	16.983	0.9982	-12.64	17.1	9.31
Krezoixm-methyl	313.35	336.2→246.2 336.2→229.2	120 120	15 15	17.644	0.9999	2.72	15.6	3.71
Penconazol	284.18	284.1→158.9 284.1→70.1	80 100	30 20	17.632	0.9999	14.48	29.2	6.23
Tebuconazole	307.80	308.1→125 308.1→70.0	100 100	25 25	17.686	0.9999	-0.05	24.5	6.94
Pyraclostrobin	387.82	388.1→194 388.1→163	100 100	10 10	17.923	0.9969	2.47	16.9	4.95
Indoxacarb	527.80	528.1→203 528.1→150	120 120	36 16	18.135	0.9954	3.15	16.0	5.37
Difenoconazole	406.27	406→337 406→251	100 100	20 20	18.202	0.9994	1.16	26.0	4.08
Quinoxifen	308.13	308→197 308→272	135 135	30 32	19.365	0.9937	11.62	13.5	7.35
Chlorpyrifos	350.59	349.9→197.9 349.9→97.0	130 130	15 41	19.469	0.9999	0.1	16.2	3.57

<sup>1</sup>MW – Molecular weight, <sup>2</sup>Frag. - Fragmentor, <sup>3</sup>CE - collision energy, <sup>4</sup>Rt – Retention time, <sup>5</sup> $R^2$  - linearity, <sup>6</sup>ME – Matrix effect, <sup>7</sup>RSD - Relative standard deviation.

The effect of matrix below 1% was recorded with carbendazim, pyrimethanil, tebuconazole azoxystrobin, metoxyfenozide and chlorpyrifos (Figure 2).

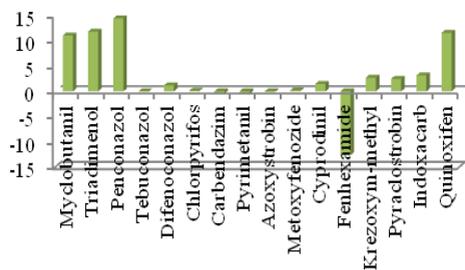


Figure 2. Matrix effect on investigated pesticides (%)

With multi-residual methods for the determination of pesticide residues the recommended interval of the recovery in the validation is from 70 to 120% (SANCO/12571/2013). By use of AC the obtained recoveries do not exceed 33% (Figure 3) which are extremely low values and point to the conclusion that AC cannot be used as a sorbent in QuEChERS method.

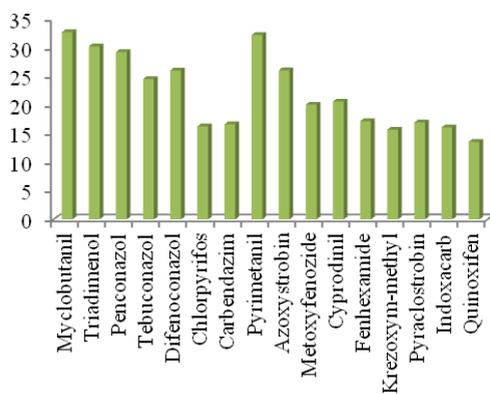


Figure 3. Average recovery of investigated pesticides (%)

## CONCLUSIONS

By use of active carbon as a sorbent aimed at the removal of planar molecules, which can affect the pesticide detection itself and their ionization in QuChERS method,

exceptionally clear and colourless sour cherry extracts were obtained. AC was excellent in reducing the matrix effect simultaneously having high  $R^2$  values ( $>0.99$ ) at the pesticide calibration.

However, this sorbent, besides being excellent at removing the impurities from the extract binds the pesticides which resulted in very low recoveries (below 33%). The obtained RSD values were below 20% which is in accordance with the valid EU regulations.

The obtained results show that the active carbon cannot be used as a sorbent in the QuChERS method and that in such a case it must be replaced by other sorbents as follows PSA, C18, GCB or Z-Sep.

## ACKNOWLEDGEMENTS

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