Application of DI-SPME/GC-MS method for the analysis of MCPA residues in winter wheat tissues

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Summary

The application of SPME/GC-MS in order to determine the (4-chloro-2-methylphenoxy)acetic acid (MCPA) residues in winter wheat seedlings tissues has been studied. The optimal conditions for the SPME of MCPA adsorption were 20 min. in 50°C and 6 min. in 220°C for desorption. The chlorophenoxy herbicide showed different level of accumulation in seedling tissues of studied winter wheat cultivars. The application of the SPME/GC-MS method for the MCPA residues monitoring and possible differences in its biodegradation in the studied wheat tissues is discussed.

Key words: chlorophenoxy herbicide residues, winter wheat, DI-SPME, GC-MS

Introduction

Recently, the use of pesticides has been increasing throughout the world. About 25% of global pesticide consumption takes place in the European Union [1]. In some EU countries the quantity of used pesticides reached almost 350 mln kg per year. Among the most popular pesticides used in Poland are phenoxy acids, initially introduced as herbicides in the late 1950s [2]. The most commonly used are (2,4-dichlorophenoxy)acetic acid (2,4-D), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) and (4-chloro-2-methylphenoxy)acetic acid (MCPA) (fig. 1).
The chlorophenoxy moiety of such herbicides contains from one to three chlorine atoms and, for MCPA, a methyl substitution. They are widely applied in the post-emergence control of annual and perennial broad-leaved weeds, cereals and grasses. As a synthetic plant growth regulators (PGRs) these herbicides are accumulated in root and stem tissues [3-5]. Since the phenoxy herbicide residues are accumulated within the cereal tissues, it is important to determine its level and duration [6]. The most common techniques are conversion of free acids to their protected ester forms (usually methyl or pentafluorobenzyl esters) and afterwards analysis with the use of GC-MS [7-8] or GC-MS-MS systems [9-10]. Moreover, determination of the pesticide residues using chromatographic techniques requires an extensive and time-consuming step of sample preparation including extraction and a clean-up procedure in order to obtain a final extract that can be used for chromatographic determination [7, 11].

![Figure 1. Structures of the basic chlorophenoxy herbicides](image)

Solid phase microextraction (SPME) appears to be a solvent-free extraction technique that presents several characteristics outlined before as a primordial phase of sample preparation technique. Nowadays, three modes of SPME can be considered: direct, headspace and membrane protected extraction. Most promising in case of extraction of the chlorophenoxy herbicide is the direct SPME extraction, where a coated fiber is directly immersed in the sample and the analytes are transported from the sample matrix (extract) to the coated fiber. In addition, the acceleration of the aqueous extraction requires an agitation [12-17].

The present paper reports on optimization of the SPME/GC-MS method for analysis of the (4-chloro-2-methylphenoxy)acetic acid residues within winter wheat tissues.
MATERIALS AND METHODS

Plants

Seeds of winter wheat *Triticum aestivum* (Roma and Sakwa cvs) were obtained from commercial store and kept at the Department of Biochemistry and Molecular Biology, University of Podlasie. They were sown into a plastic pots filled up with medium soil and germinated in an environmental cabinet (21°C, relative humidity 70%, 16 h of daylight and 8 h of darkness). The experimental pots were regularly watered and no extra fertilizers were applied. Ten days old seedlings of the winter wheat Roma and Sakwa cvs were sprayed out with three doses of Chwastox 300 SL (MCPA): 600 g/l, 300 g/l, 150 g/l. Then the seedlings were collected after 1, 4, 8, 12, 24 h and after 2, 3, 4, 5 days.

Chemicals

The reagents: 99.5% methanol, 99.5% acetone, 99.9% dichloromethane (Sigma), standard methyl-4-chloro-2-phenoxyacetate 99.5% (Supelco), commercial preparation of Chwastox Extra 300 SL, containing 30% of MCPA as a sodium salt and 70% of non-specified ingredient were purchased in Organika Sarzyna (Nowa Sarzyna, Poland). All chemicals were stored in darkness at 4°C.

Extraction of the herbicide residues from plant tissues

The sample of about 30 g of plant tissues was shaken with mixture of acetone-NaCl-water (3:1:6 v/v) for 15 min, and then filtered off with a Whatman No 1 filter paper. Then the dichloromethane extraction of plants filtrates was performed and the extracts were evaporated to dryness at a temperature of 35°C using a rotary evaporator. The residue was redissolved in 1 ml of methanol, and then the solid phase microextraction was performed.

SPME procedure and optimization process

The CAR/PDMS fiber in the thickness of 75 µm was conditioned before initial application into injection port of the gas chromatograph by heating at 280°C for 1 h. Then the fiber was exposed to the stirred sample with addition of 0.5% NaCl for an adsorption (extraction range 5–30 min. at a temperature of 20–70°C). When the adsorption was completed the fiber was removed from the sample and introduced into the GC injector where the thermal desorption of the analytes (desorption range 2–10 min. at a temperature of 140–240°C) was carried out. The procedure optimization process was carried out using 10 µg/l internal standard methyl-4-chloro-2-phenoxyacetate.
GC-MS analysis

The GC-MS analysis was performed on a Shimadzu series GC 17A gas chromatograph equipped with split/splitless injector and interfaced with QP-5050 Shimadzu mass spectrometer. The GC was equipped with capillary column BPX-5 (Phenomenex) (30 m x 0.25 mm I.D. with 0.25 µm film thickness) connected to the split/splitless injector. The optimized oven temperature program was at first 80°C (5 min.), then from 80°C to 280°C (at 20°C/min). The final temperature was held for 5 min. A column head pressure was 56.7 kPA and an injector temperature was 220°C. Helium was used as the carrier gas at a flow rate of 9.8 ml/min.

The mass spectrophotometer was operated in the electron impact ionization (EI), and the applied energy was 70 eV. Mass spectra were acquired in the mass range from m/z 50 to 450. The detection of MCPA was also accomplished in selected ion monitoring (SIM) mode, using the following fragment ions: m/z 141, 155, 214 (methyl-4-chloro-2-phenoxyacetate). The instrumentation was controlled by software CLASS 5000 with NIST107 and NIST21 library of mass spectra.

RESULTS

The carried out analysis showed the presence of the methyl-4-chloro-2-phenoxyacetate within the studied wheat tissues. After GC-MS separation, the methyl-4-chloro-2-phenoxyacetate showed a single peak that was identified after the comparison of MS spectrum these herbicide and NIST spectrum library [18] (fig. 2).

The obtained results showed the highest adsorption of the MCPA on the CAR/PDMS fiber after 20 min. at 50°C, while the adsorption procedure was optimized. The optimal desorption conditions for the studied analytes from the coating fiber were found after 6 min. at the temperature of 220°C. The data in the table 1 showed basic statistical parameters of the optimization procedure.
Optimization parameters of the ΔI-SPME/GC-MS of the methyl-4-chloro-2-phenoxyacetate

<table>
<thead>
<tr>
<th>parameter</th>
<th>optimization range</th>
<th>LOD [g/l]</th>
<th>MC [g/l]</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>adsorption time</td>
<td>5-30 (min)</td>
<td>0.003590</td>
<td>0.011690</td>
<td>1</td>
</tr>
<tr>
<td>adsorption temperature</td>
<td>2-70 (°C)</td>
<td>0.002400</td>
<td>0.029690</td>
<td>1</td>
</tr>
<tr>
<td>desorption time</td>
<td>2-10 (min)</td>
<td>0.003702</td>
<td>0.014740</td>
<td>1</td>
</tr>
<tr>
<td>desorption temperature</td>
<td>140-240 (°C)</td>
<td>0.000005</td>
<td>0.011240</td>
<td>1</td>
</tr>
</tbody>
</table>

LOD – limit of detection; MC – maximum concentration; RSD – research standard deviation

Residues of the studied herbicides were present in the tissues of Sakwa cv. only until 12 h after treatment. After that time the level of the MCPA was undetectable. During first 8 h from spraying the content of the herbicide that occurred in tissues of this cultivar was strictly related to the applied dose. Thus, when the dose of 600 g/l was applied the highest accumulation of the MCPA was found and when lowest concentration (150 g/l) of the herbicide was applied the lowest level of the residues occurred in the wheat seedlings (fig. 3).

![Figure 3. Accumulation of the (4-chloro-2-methylphenoxy)acetic acid residues in Sakwa cv. tissues after spraying with various doses of the herbicide](image-url)

Similar dose-dependent tendency was observed when the accumulation of MCPA was studied in seedlings of the Roma cv. However, in tissues of this wheat cultivar the herbicide residues were present for much longer time, up to three days. In all performed experiments the level of the residues declined gradually. After four days in the Roma cv. tissues the level of the MCPA was undetectable (fig. 4).
**DISCUSSION**

The experiments carried out showed a high potential of this microsampling technique for the analysis of the chlorophenoxy herbicides. In the performed DI-SPME study the optimal fiber exposition to the stirred sample was found to be 20 min. at the temperature of 50°C under perfect agitation conditions. The time required for reaching equilibrium is significantly lower as compared to the same experiment performed at static (with no agitation) conditions. After that, the equilibrium time (20 min.) between fiber coating and aqueous phase the heating of the sample might probably result in the loss of precision [16]. The best conditions for desorption of the studied herbicides were found when the sample was held at the temperature of 220°C for 6 min. On the other hand, the desorption temperature 250 °C was optimal for chloroorganic insecticides [19, 20], dirone [21], [19, 20, 22] and other pesticides [23]. The obtained optimal desorption time (6 min.), was similar to that found for atrazyne [25], triazyme [22] and other pesticides [19-21, 25-27].

In the performed experiments there was found a much higher concentration of (4-chloro-2-methylphenoxy)acetic acid (MCPA) residues in seedlings of the Roma cv. than in tissues of the Sakwa cv. Interestingly, the duration of biodegradation of the MCPA in Roma cv. was six-fold longer than in Sakwa seedlings. The question if it was due to various mechanisms of biodegradation is still open. Accordig to Grossman and Scheltrup [28], grass tissues sprayed with quinclorac (ACC) showed the highest concentration of these herbicide in root tissues 3 h after treatment.
On the other hand, 24 h after treatment about 5% of the herbicide residues was transformed into polar metabolites. Lichtenstein and Shultz [29] affirmed that the level of the pesticide accumulation varied even between single plants of the same species. Such situation was documented for cultivars of *Daucus carota* where after root treatment with aldrine and hetpachlore different levels of accumulation of these herbicides were found. According to Harborne [30], cereal tissues have a high ability to intensive herbicide metabolism in comparison to weed species [31]. The biodegradation of chlorophenoxy herbicides usually depends on binding glucose or aspartic acid to carboxylic groups of these herbicide and on hydroxylation of their aromatic rings [32-33].

**CONCLUSIONS**

Solid phase microextraction (SPME) followed by gas chromatography-mass spectrometry offers a very sensitive and precise method for analysis of (4-chloro-2-methylphenoxy)acetic acid residues in plant tissues. Moreover, SPME/GC-MS reduce the amount of organic solvents usage and shorten the time of the analysis.

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**REFERENCES**

ZASTOSOWANIE DI-SPME/GC-MS DO ANALIZ POZOSTAŁOŚCI KWASU MCPA W TKANKACH PSZENICY OZIMEJ

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Streszczenie

Zbadano możliwość zastosowania metody SPME/GC-MS do analiz pozostałości kwasu 4-chloro-2-metylofenoksyoctowego (MCPA) w tkankach siewek pszenic ozimych. Stwierdzono, że w przypadku adsorpcji optymalne warunki dla mikroekstrakcji SPME estru metylowego MCPA to temperatura 50°C i czas trwania procesu 20 min, a dla desorpcji odpowiednio 220°C i 6 min. W tkankach siewek pszenic ozimych stwierdzono zróżnicowaną zawartość pozostałości MCPA. Prawdopodobnie mechanizmy biodegradacji tego herbicydu są zróżnicowane.

Słowa kluczowe: pozostałości herbicydów chlorofeonoxyoctowych, pszenica oziom, DI-SPME, GC-MS